



Use of DNA Microarray Analysis to Identify Genes and Signaling Pathways Central to the Anti-Melanoma Effect of IFN- γ

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Interferon and Melanoma

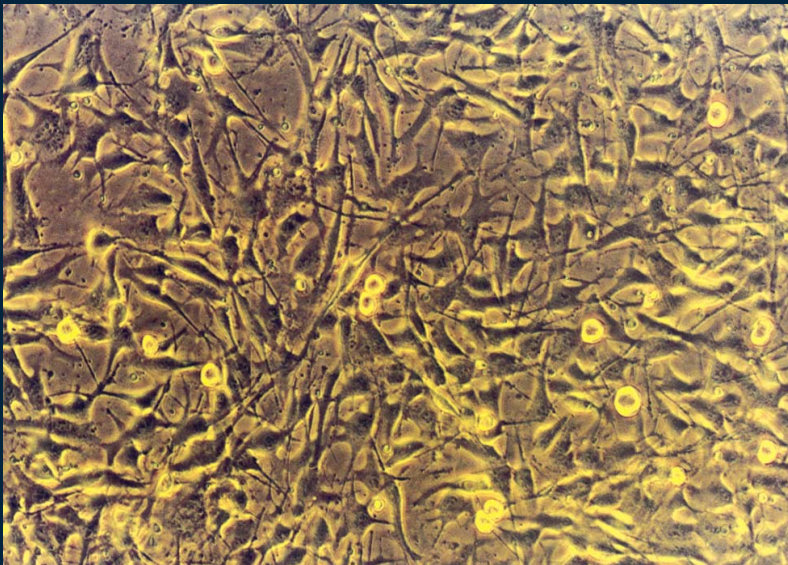
- Type I and type II interferons have direct static and/or cidal effects on only a small fraction of melanoma cell lines, even though most cell lines express IFN receptors and activate the Jak/Stat pathway in response to IFN- α and IFN- γ
- Clinical response rate of melanoma to IFN- α is 10-15%; few responses seen in old trials of IFN- γ alone
- No evidence that antitumor effect of IL-2 or interferons involves specific antitumor immunity
- Evidence that cellular response to interferons may play role in preventing tumorigenesis:
 - IFN- γ K.O. mice develop lymphomas and solid tumors
 - Malignant transformation of Li-Fraumeni fibroblasts and transition from melanocyte to melanoma associated with downregulation of IFN response genes

Effects of Interferons on 3 Human Melanoma Cell Lines

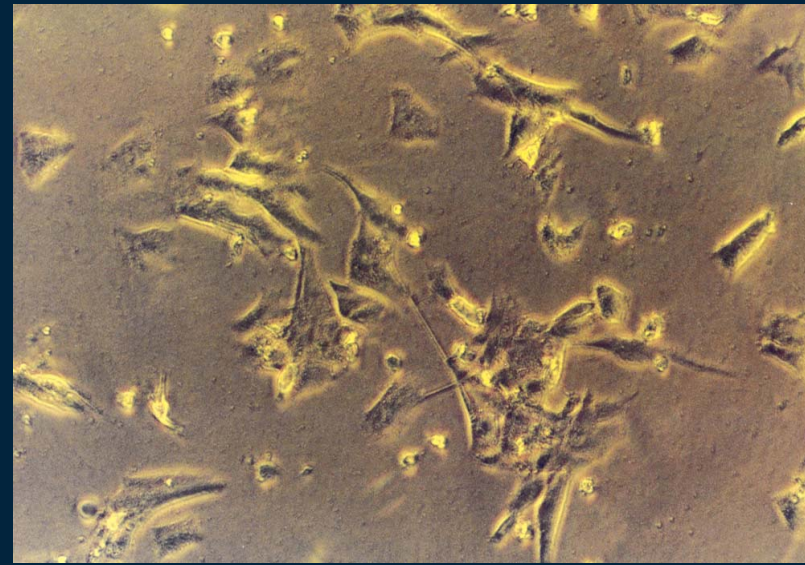
	<u>Pro-Apoptotic</u>			<u>Anti-Proliferative</u>		
	DM6	DM93	501mel	DM6	DM93	501mel
IFN- γ	+	-	-	+	-	-
IFN- α	-	-	-	-	-	-

Effect of IFN- γ on DM6 Melanoma

Control



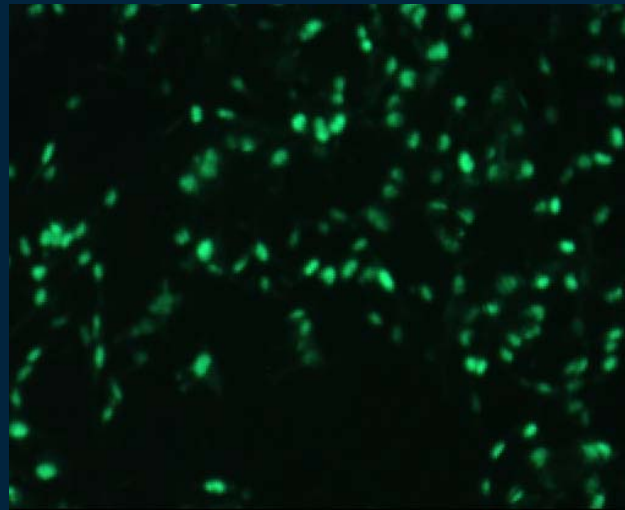
IFN- γ



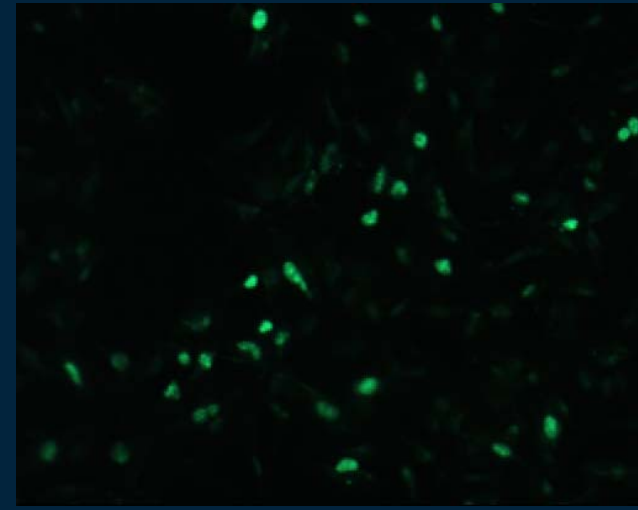
Antiproliferative and Proapoptotic Effects of IFN- γ in DM6

Ki67

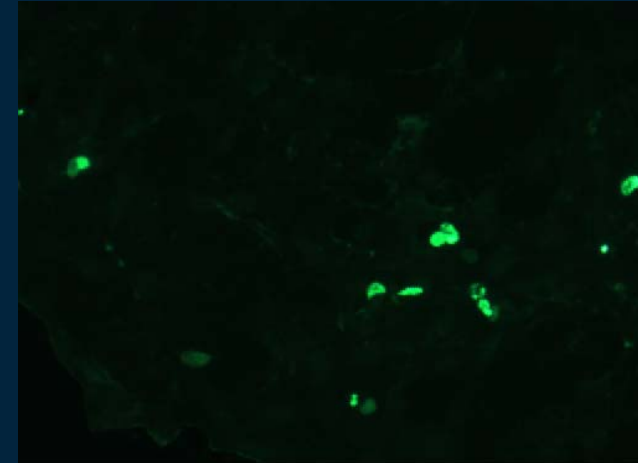
Control



IFN- γ



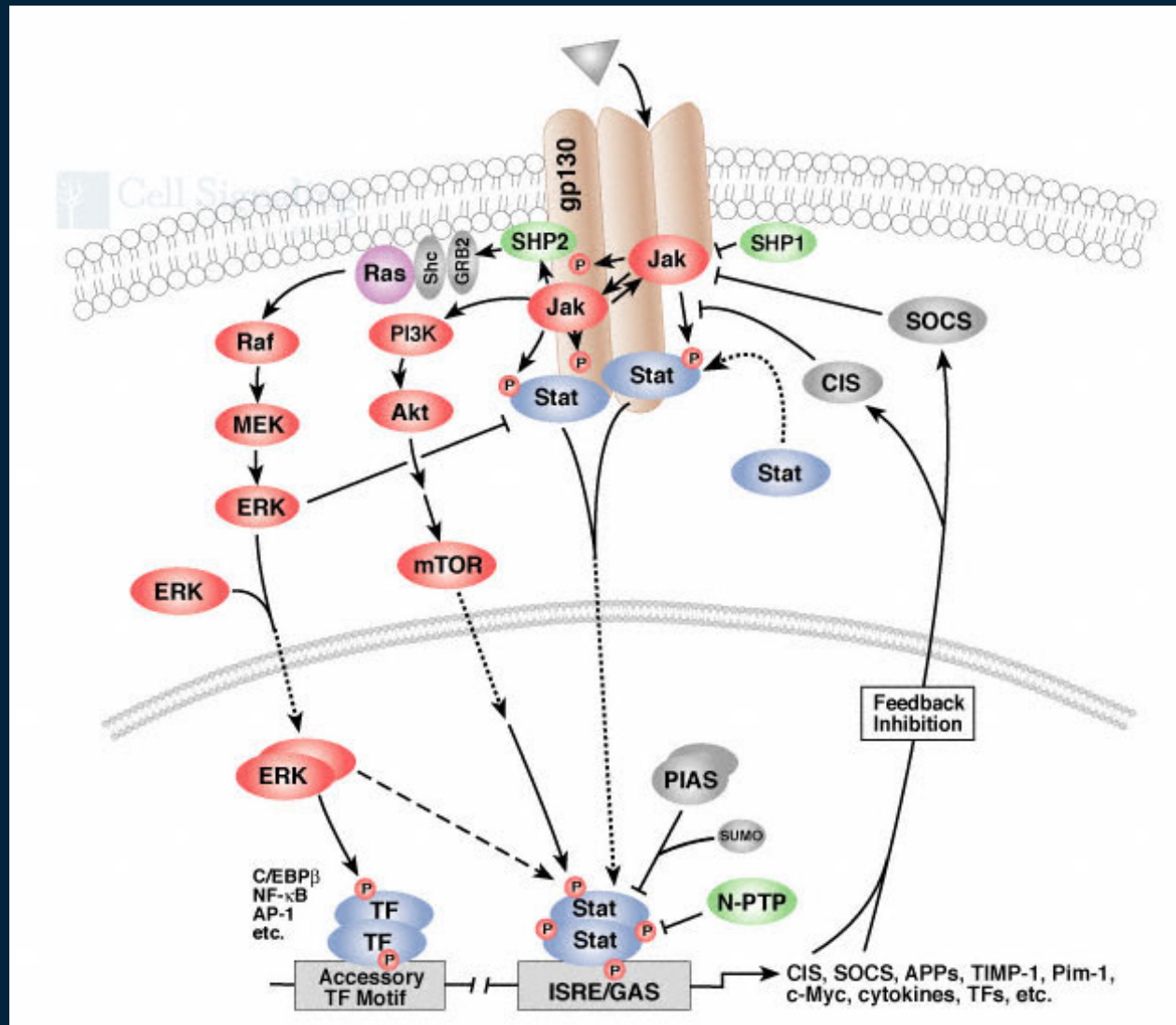
TUNEL



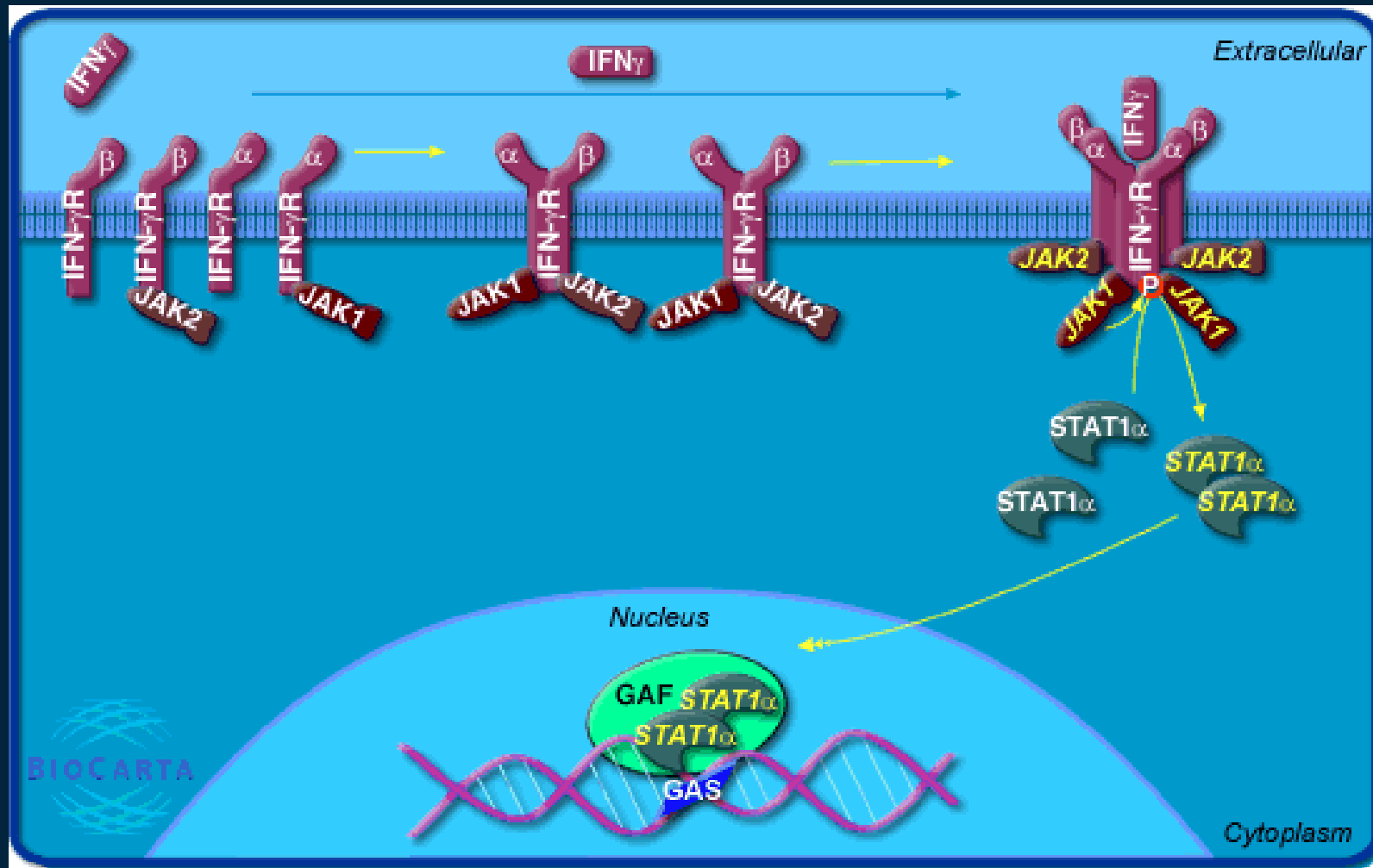
Direct Effects of Interferons on Melanoma: Questions

- Which signaling pathways mediate the direct pro-apoptotic and antiproliferative effects of IFN?
- Do interferons alter the expression of certain key genes to achieve these effects?
- What is the molecular basis for resistance of melanoma to these effects of interferons, and can this resistance be overcome?

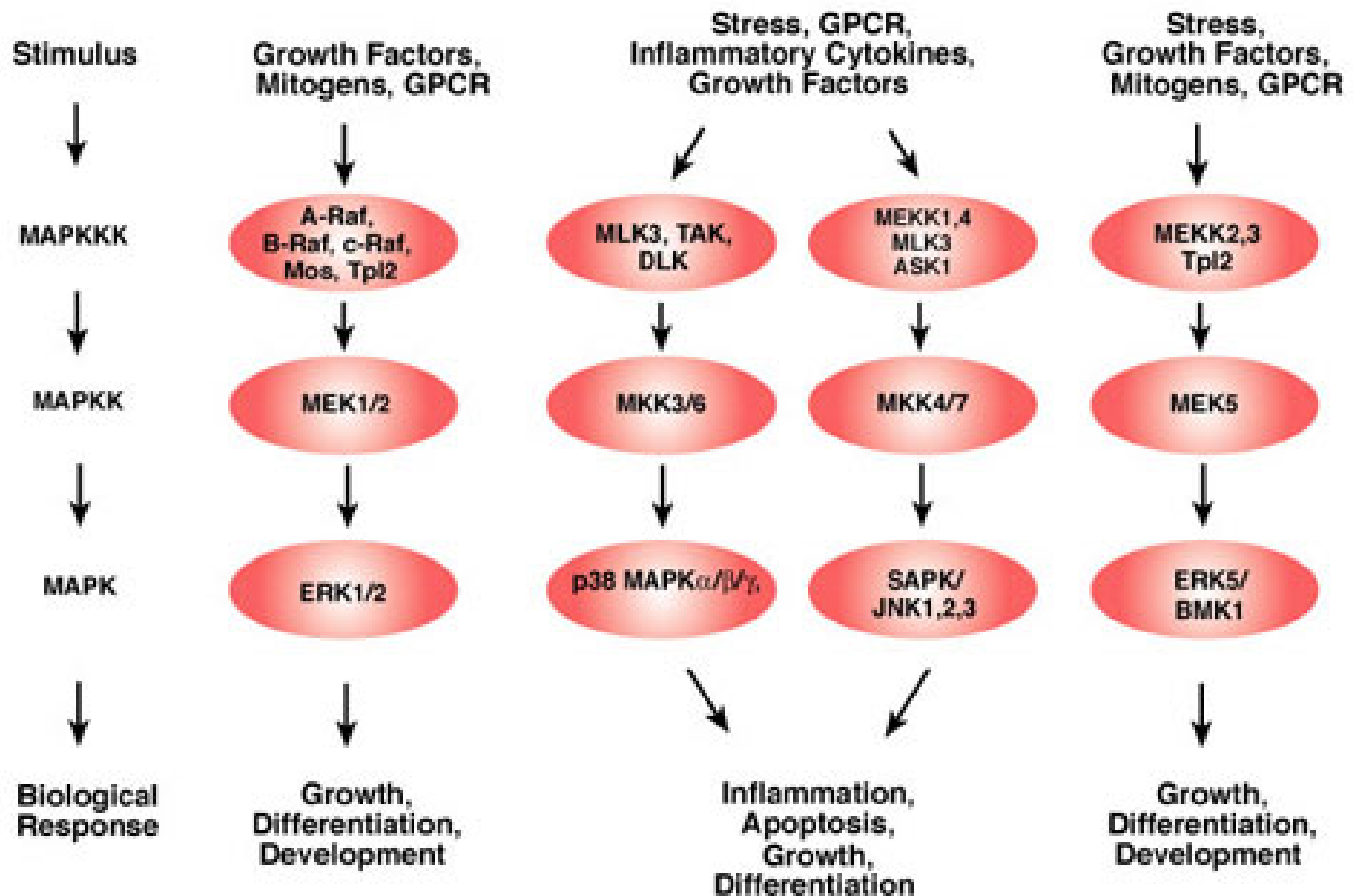
Cytokine Receptor Signaling Pathways



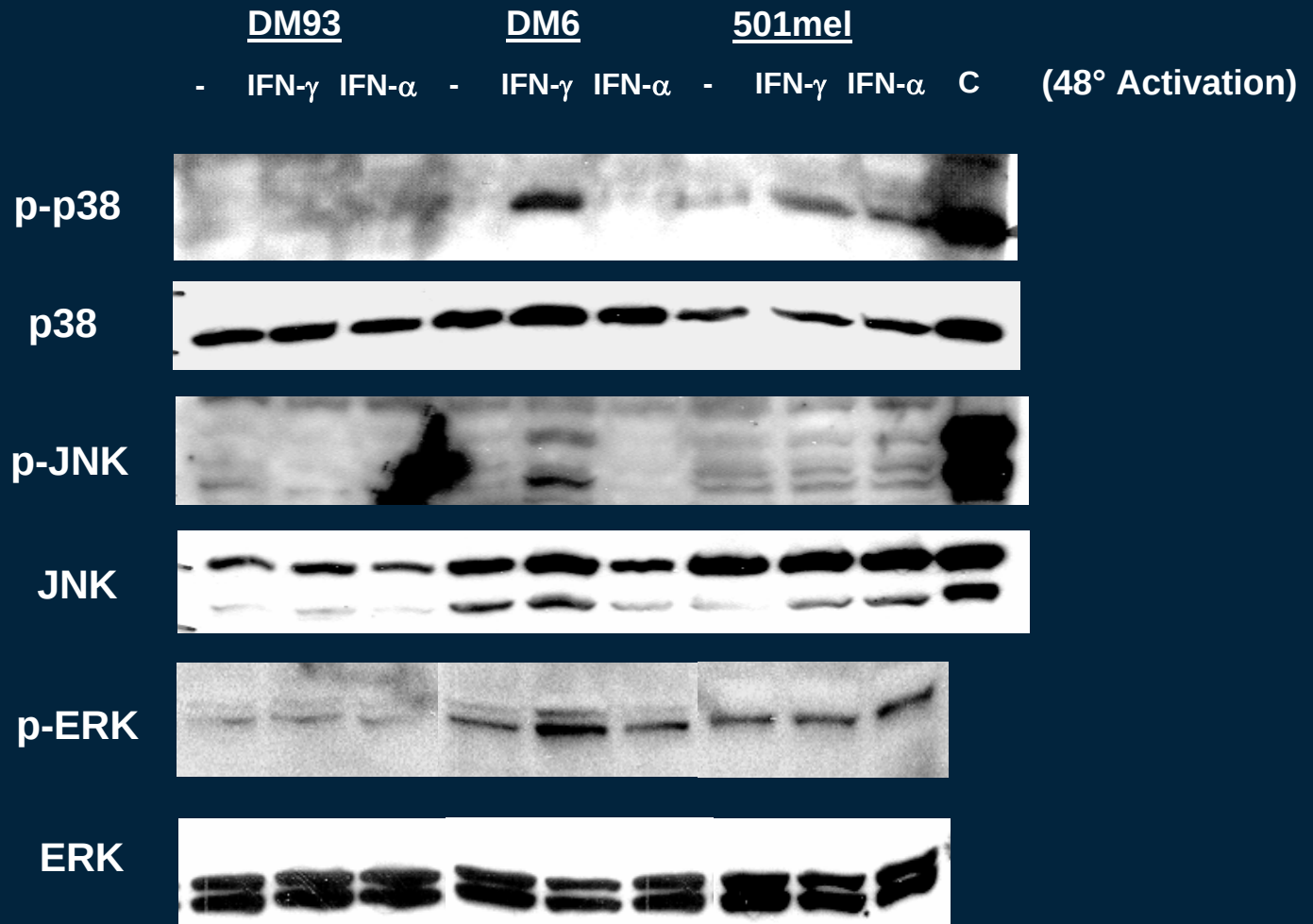
Interferons and Jak/Stat Signaling



MAP Kinase Signaling Pathways



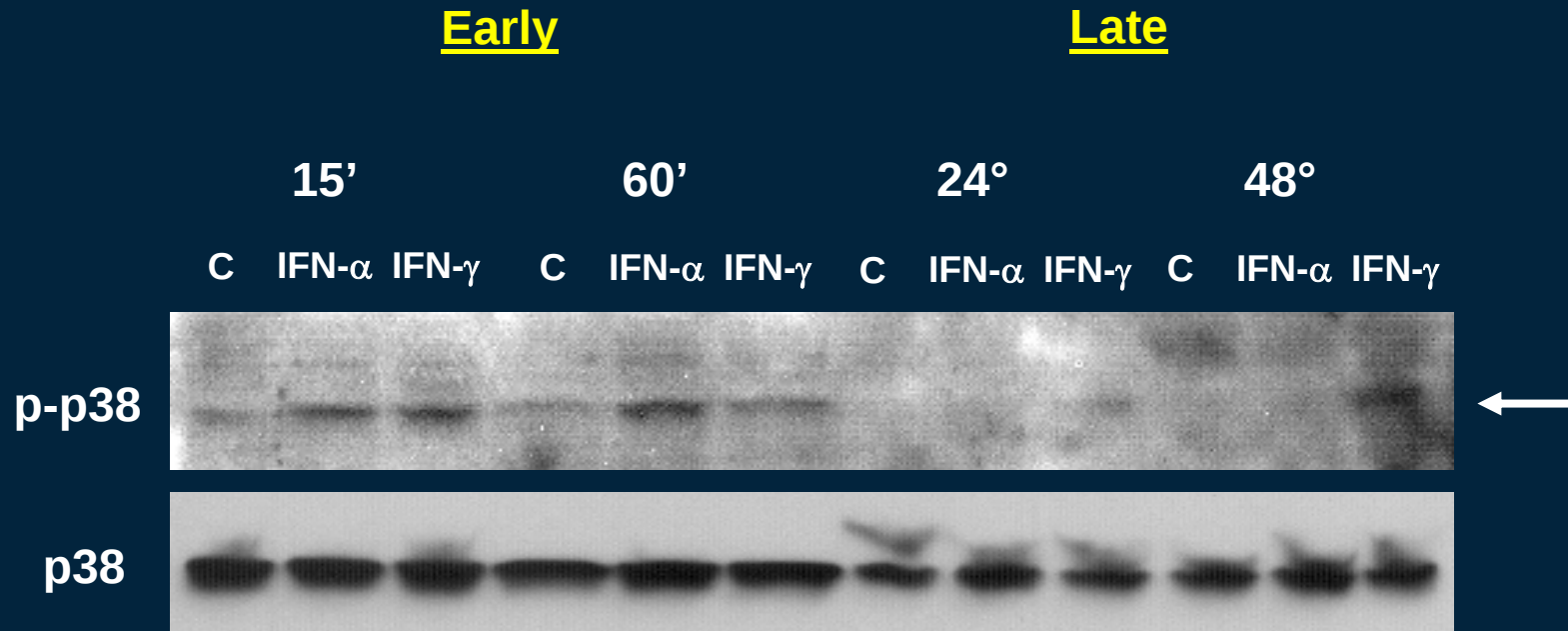
IFN- γ Activates MAPKs in DM6



Late

p-ERK

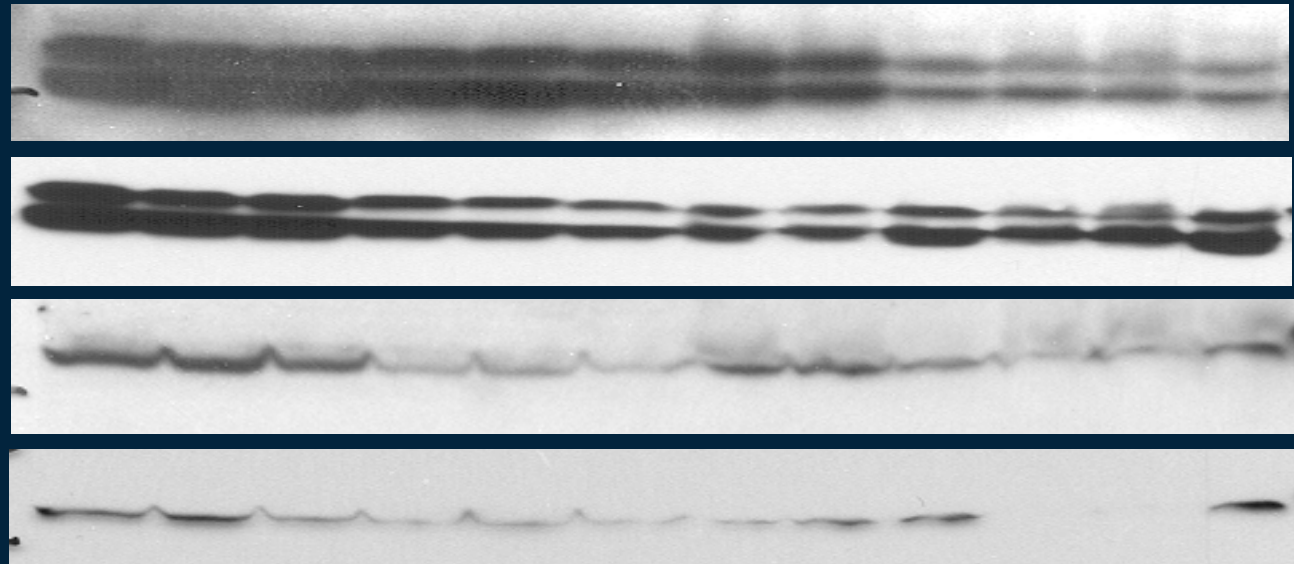
Biphasic p38 Activation By IFN- γ in DM6



Late

48°

p38

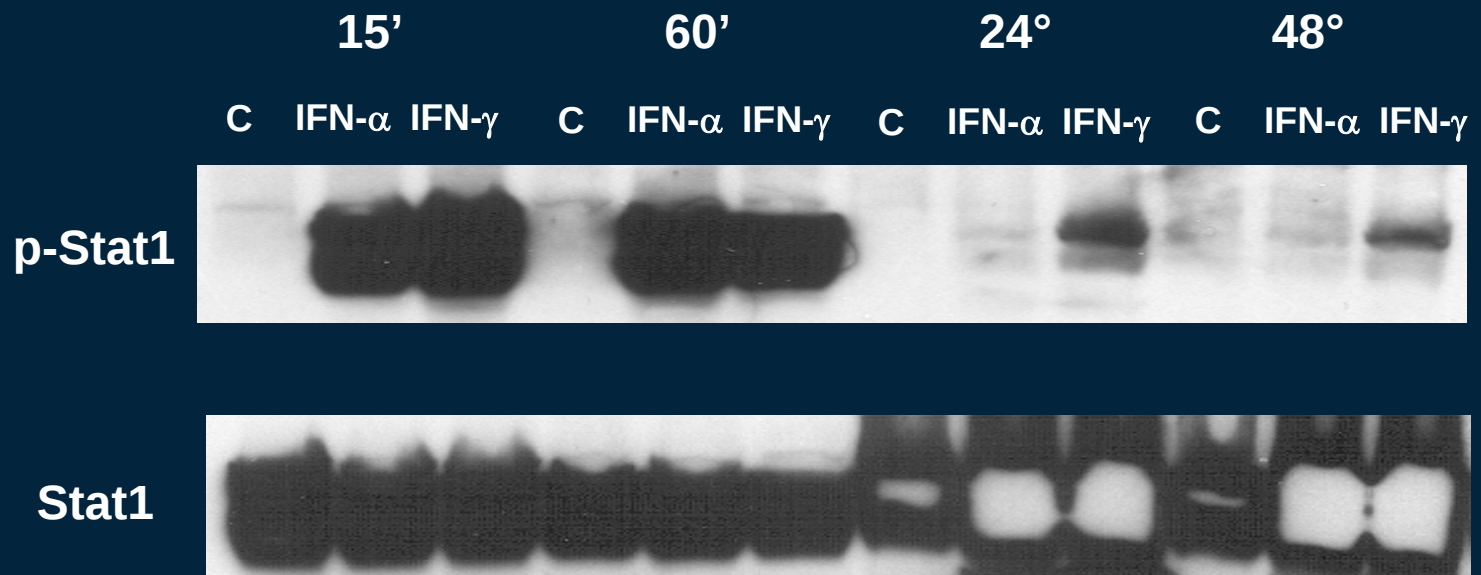


Early Phase MAPK Activation By Interferons

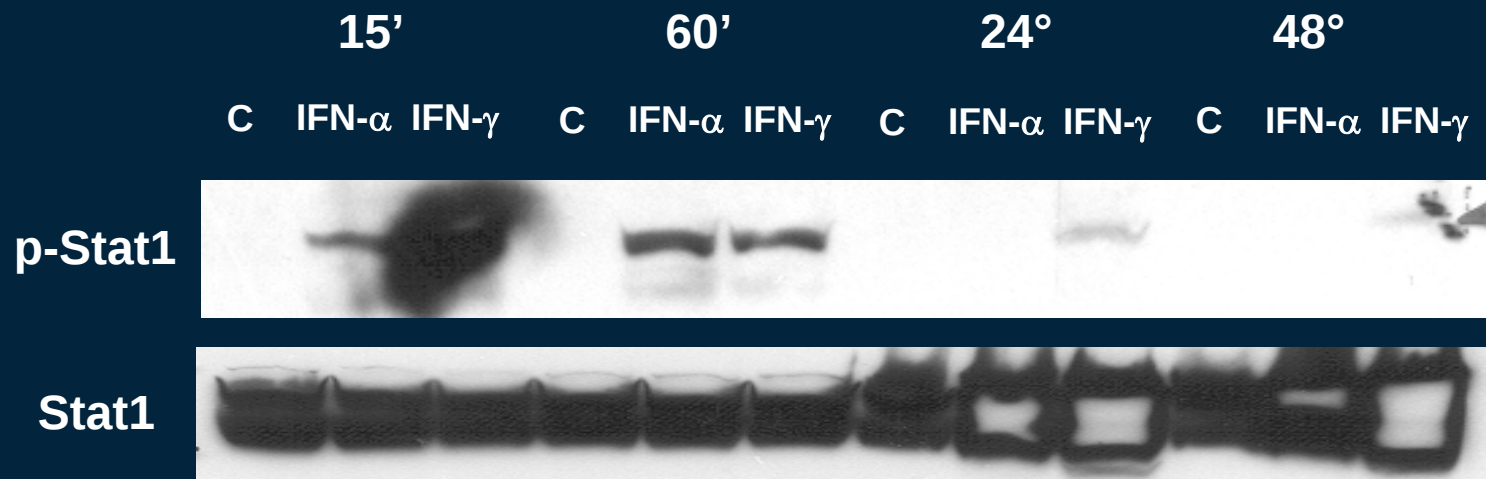
	<u>ERK</u>			<u>p38</u>			<u>JNK</u>		
	DM6	DM93	501mel	DM6	DM93	501mel	DM6	DM93	501mel
IFN- γ	+	-	-	+	-	-	+/-	-	-
IFN- α	+	-	-	+	-	-	+/-	-	-

[illegible]

Monophasic Stat1 Activation By IFN- γ in DM6



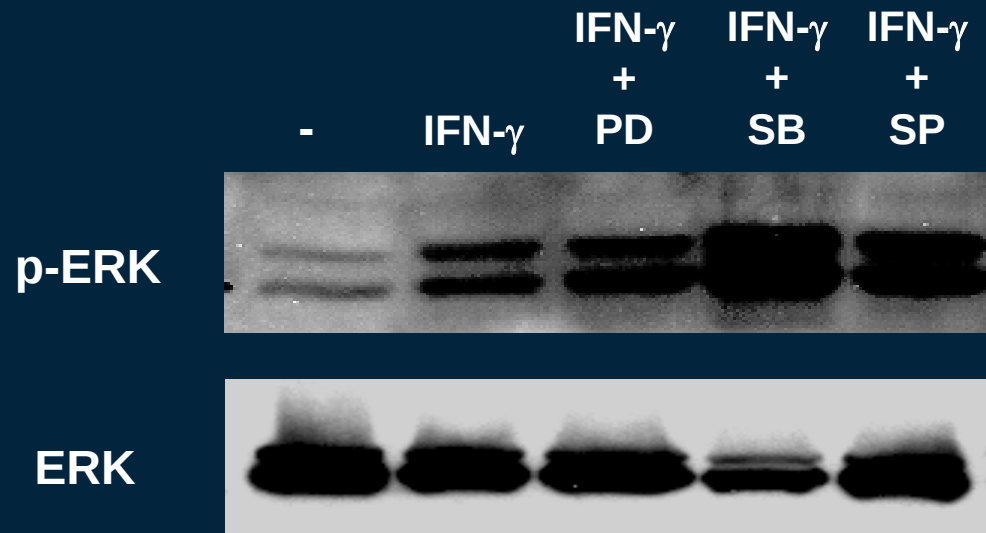
Monophasic Stat1 Activation By IFN- γ in 501mel



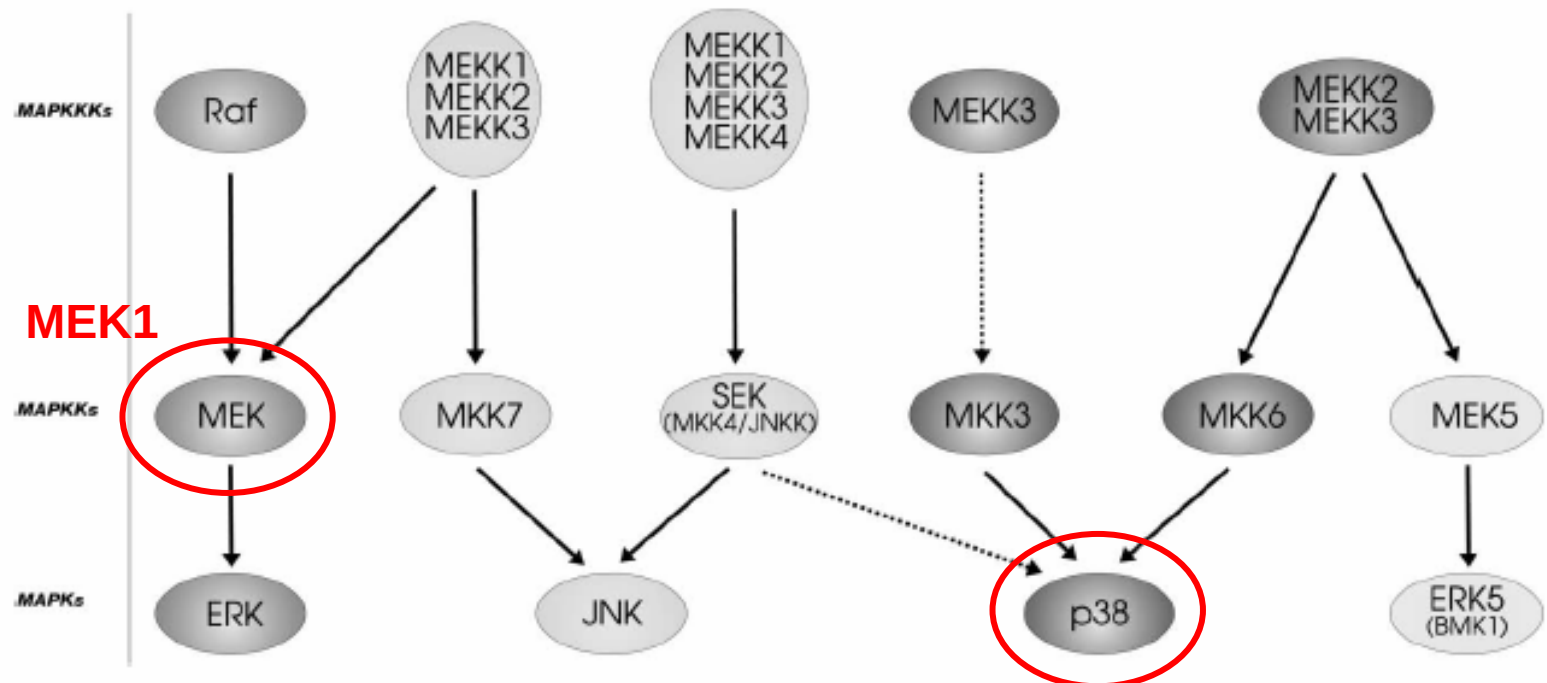
MEK1 or p38 Inhibition Blocks Effect of IFN- γ on DM6

	IFN- γ	IFN- γ + PD (MEKi)	IFN- γ + SB (p38i)	IFN- γ + SP (JNKi)
Pro- apoptotic	+	-	-	+
Anti- proliferative	+	-	-	+

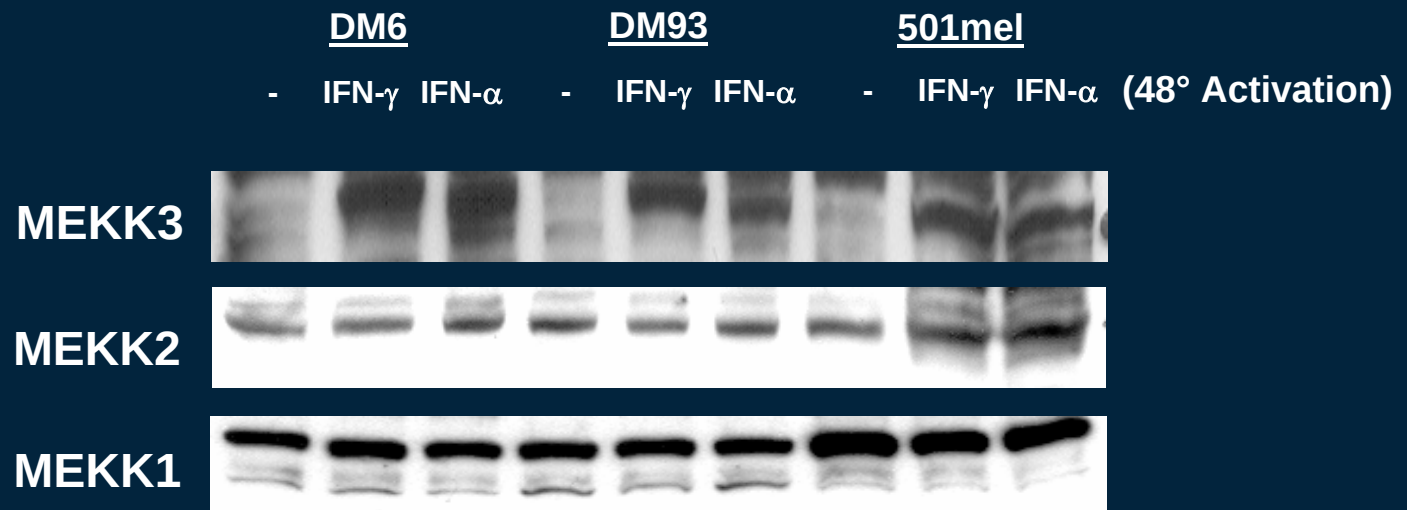
MEK1 Inhibitor Does Not Block ERK Activation By IFN- γ in DM6



Anti-Melanoma Effect of IFN- γ Associated with MEK1 and p38 Activation

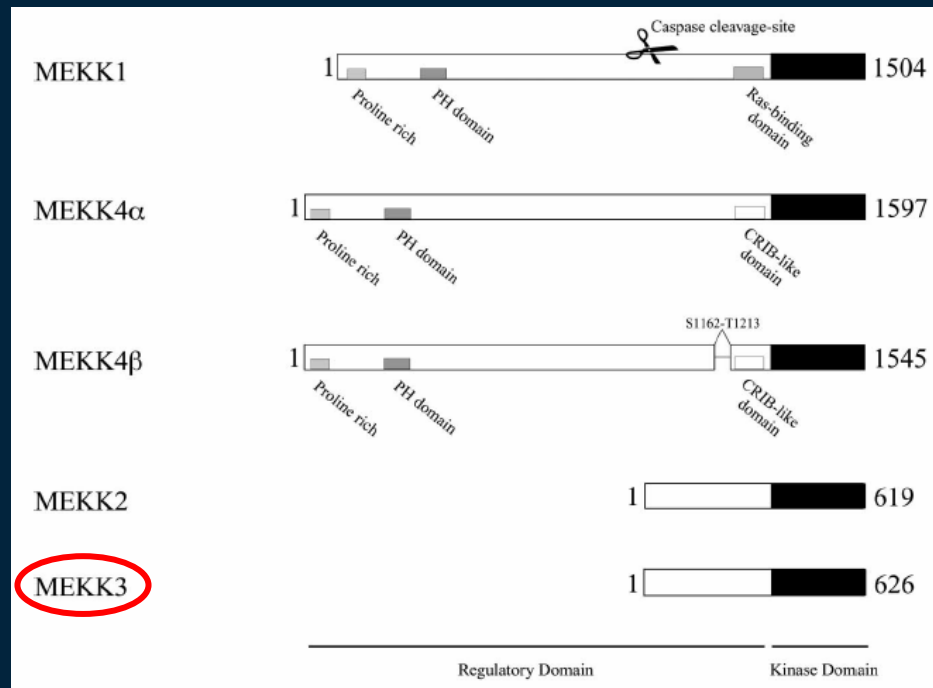


MEKK3 Expression, But Not MEKK1 or MEKK2, is Modulated By Interferons in DM6, DM93 and 501mel

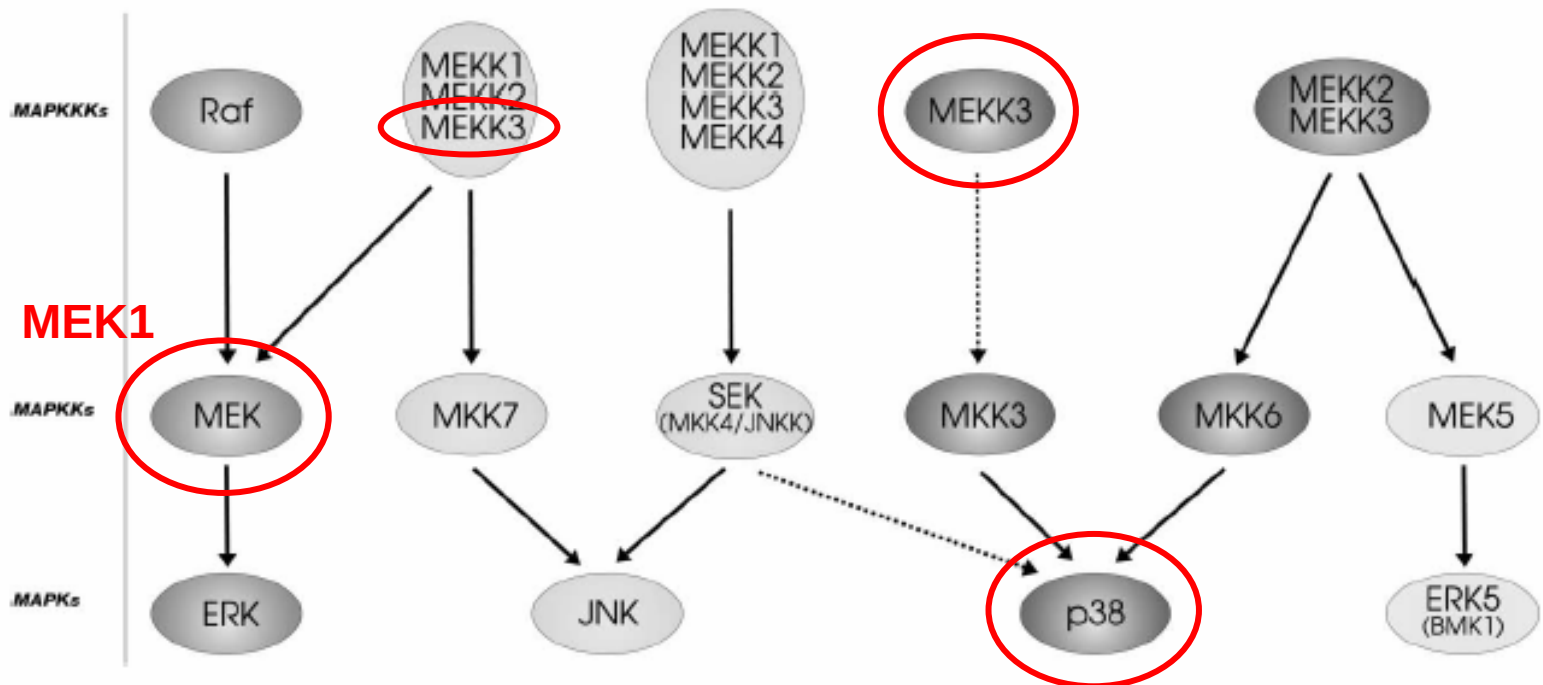


MEKK3

- Serine-threonine kinase
- Conserved kinase domain
- Small regulatory domain
- Activation modified through interaction with:
 - BRCA1
 - TRAF7



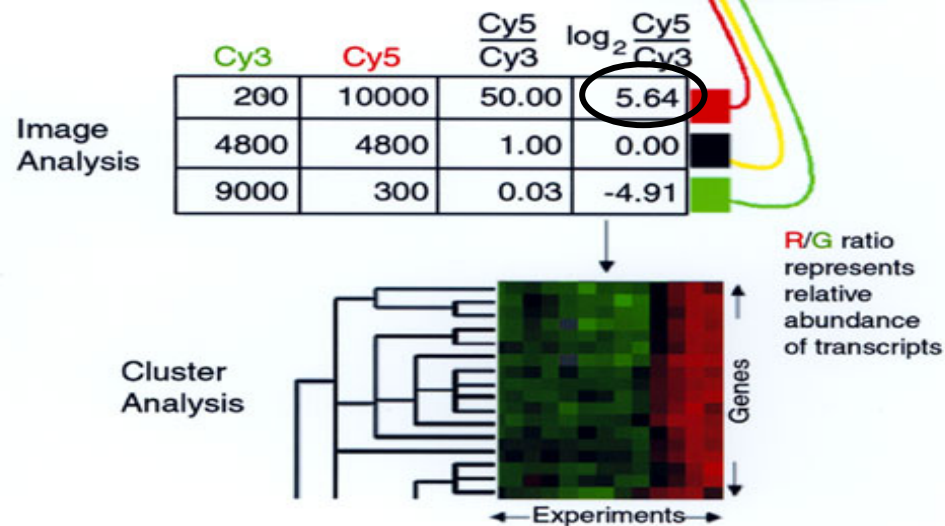
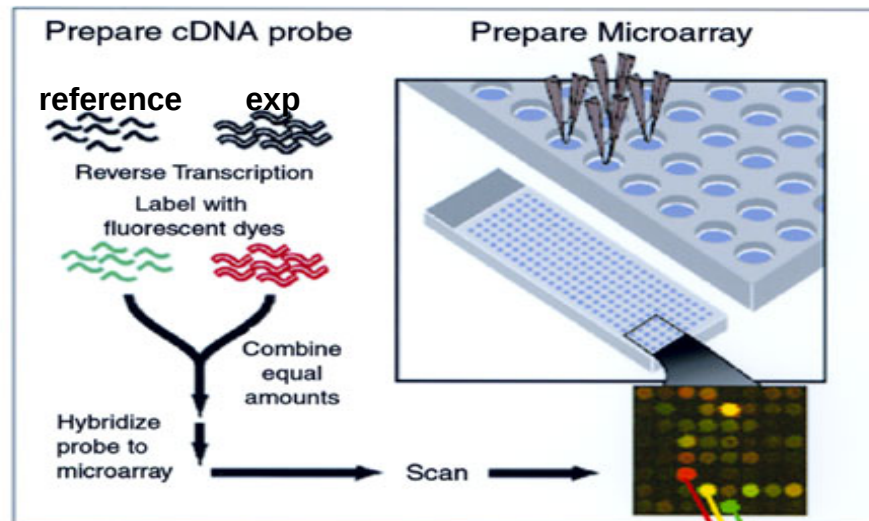
Induction of MEKK3 By IFN- γ : Possible Role in Late Phase of MEK1 and p38 Activation

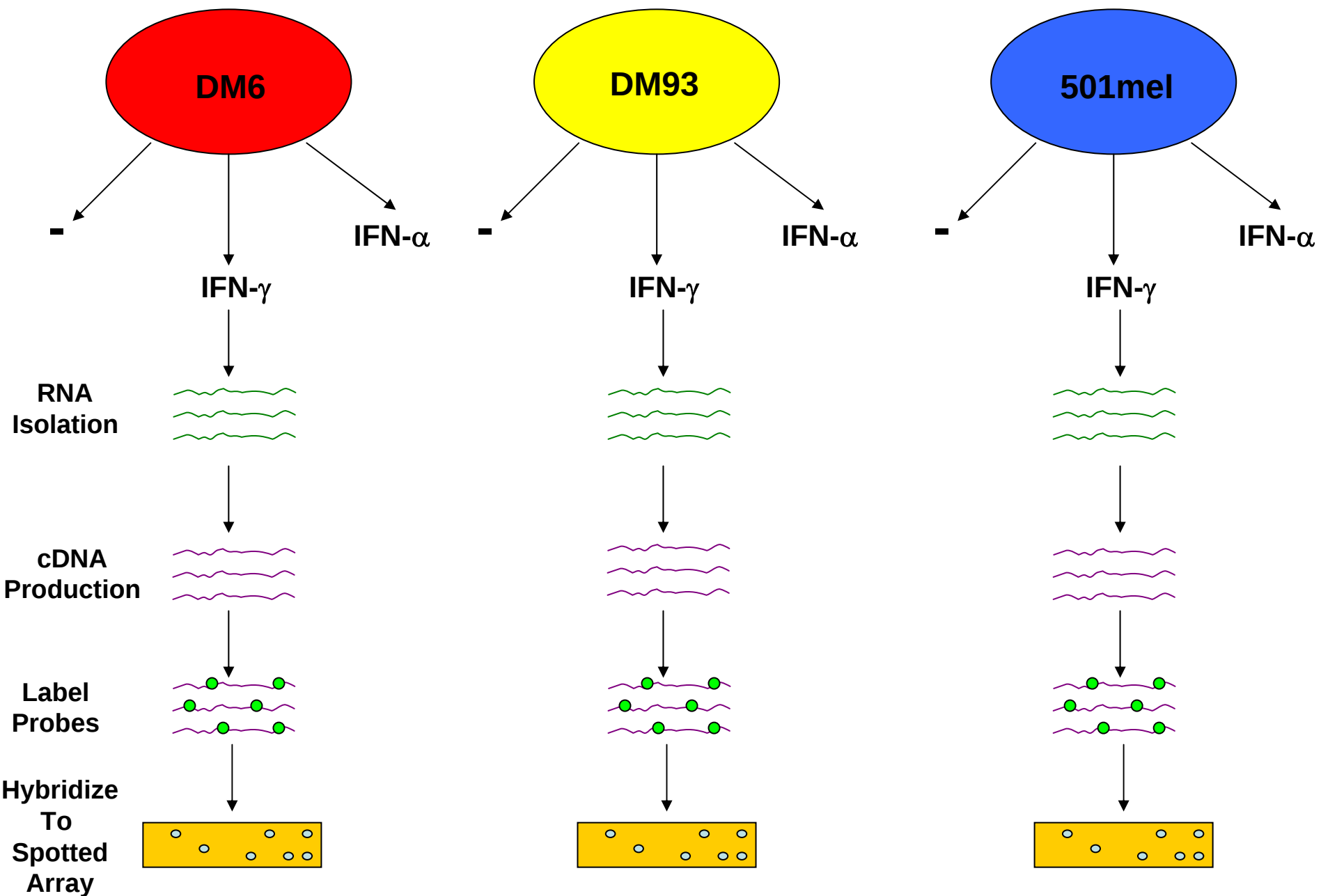


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Spotted DNA Microarray





Number of Genes Changing 2-fold or More Over Baseline ($P < .05$) in Response to Interferons

	DM6	DM93	501mel
IFN- α only	17	462	45
IFN- γ only	1085	0	167
IFN- α + IFN- γ	475	45	722

Genes Downregulated Exclusively By IFN- γ in DM6 Alone

DM6			DM93			501mel			GENE	
-	IFN- γ	IFN- α	-	IFN- γ	IFN- α	-	IFN- γ	IFN- α		
3.327802	1.337344	4.40229	4.630206	3.411458	2.266255	10.25511	9.125445	9.461002	RAB27A	← G-protein
2.085755	0.814269	1.678545	2.967095	3.407176	1.784451	2.489174	3.483816	4.442175	MGC4840	
2.942937	1.13515	2.958132	6.824674	5.364951	3.745075	1.632116	2.358373	3.503117	FLJ20716	
7.124522	2.711024	5.029543	5.02557	4.486624	3.154044	8.431795	8.185545	8.069087	TC10	
4.259401	1.619702	3.238065	10.74748	11.41523	8.547368	17.77577	14.94852	20.77267	RAB38	← G-protein
3.147678	1.193258	3.051527	2.917824	3.279179	2.771399	5.2112	4.350074	5.763292	STX7	
4.54684	1.701478	5.530992	8.649982	5.120966	3.906772	8.846674	8.553221	11.2603	AP1S2	
2.157577	0.7941	3.200794	4.587723	2.88094	2.004122	1.754995	2.134409	2.134426	SGK	
2.796748	1.01763	2.749528	3.184366	2.175445	2.513753	4.483355	2.360925	1.928405	LOC51118	
3.202132	1.117286	4.191283	16.02791	14.52414	8.895234	14.65851	11.90486	14.72676	ITGA7	
5.943004	1.963225	4.183504	4.930815	4.609768	4.135738	3.901563	3.610336	5.501083	KIAA0608	
2.262259	0.721638	2.954657	3.497574	2.775334	2.320025	2.180088	2.206042	2.421924	DKFZP762	
4.279099	1.347793	4.018971	6.54117	6.838704	5.700484	4.784775	6.618671	6.234479	ENDOFIN	
6.948993	2.04636	6.017687	9.369288	7.083647	4.596342	4.849374	5.075257	8.421149	ASAH	
5.716741	1.656401	4.31598	4.269292	7.274527	3.629205	4.847324	6.788366	7.201003	CA14	
8.057691	2.328387	5.736601	5.96542	6.2279	4.592017	7.655421	8.671587	9.771288	TNFRSF14	
4.342189	1.096025	3.808344	3.152763	3.443113	2.008378	2.921768	3.175827	3.222199	RAGD	← GTP-binding protein
4.567463	1.07453	7.454314	12.63644	10.21938	5.269111	10.7701	11.21371	9.72658	QPCT	
5.095446	1.16165	3.182604	5.839951	6.179755	4.946895	6.382297	7.433513	8.064543	GPRC5B	← G-protein coupled receptor
4.838039	1.070657	9.368489	7.352964	6.083609	3.88642	5.33786	4.334049	4.791175		
3.867728	0.854473	3.121408	5.820447	4.280983	2.7938	2.449297	2.716387	3.278403	EDNRB	
3.610351	0.740077	6.316348	4.407205	3.796506	1.989775	6.124313	8.194269	7.201849	FLJ10633	
3.276365	0.658	2.135642	1.657239	1.41646	1.518033	14.54726	16.72738	21.53263	GSTP1	← Drug resistance
7.768647	1.554047	8.189777	5.508492	5.451184	4.878237	4.043445	5.044092	5.124356	LGALS3	← Anti-apoptotic
2.984595	0.593587	3.182802	2.467421	2.085365	1.292013	1.566056	1.322698	1.536005		
3.776842	0.724945	2.069774	2.378315	2.213384	1.944656	4.323429	3.482201	3.58261	RAP2B	← GTP-binding protein
3.548298	0.622736	4.173765	2.894101	3.486593	1.594192	1.5427	1.451356	2.126677	TNFAIP3	
14.41956	2.518126	15.41603	13.67877	10.78718	5.830556	15.94623	20.70527	18.20734	CAPN3	← G-protein signaling
3.299246	0.552032	2.561536	2.020979	1.989628	1.422174	0.908081	0.611455	0.749635	FLJ10493	
6.645189	1.107982	3.851912	4.926597	4.835573	4.368935	6.129077	5.972393	7.200931	GPR56	← G-protein coupled receptor
4.647002	0.734737	6.925034	3.988629	3.495535	2.150182	4.854728	4.484916	4.10529	CDK2	← Cell cycling
6.280252	0.77458	4.343436	4.449734	3.344997	1.979113	5.979087	5.509162	6.787427	CD68	
12.01272	1.425875	29.32951	19.01999	17.92946	5.303856	15.00176	12.56215	13.11734	MLANA	
5.296591	0.60825	5.030192	4.206006	6.882648	4.90096	7.42248	13.3034	14.74419	CDH3	← P-Cadherin
11.15975	1.259888	8.772902	6.87637	3.78946	3.881819	18.95021	9.495471	23.34503	SLUG	← Transcriptional repressor
14.39765	1.554904	13.80859	4.621219	4.794604	3.227989	32.90468	28.49208	29.07462	DKFZP761	
12.38419	1.211673	10.24103	2.438998	2.404036	1.488415	22.542	32.70683	29.05211	BCL2A1	← Anti-apoptotic

Genes of Interest Downregulated By IFN- γ in DM6

- **G-Protein Signaling**
 - RAB27A, RAB38, RAGD, Rap2B
 - GPRC5B, GPR56
 - Calpain (CAPN3)
- **Apoptosis Inhibitors**
 - LGALS3
 - BCL2A1
- **Transcription Factors**
 - SLUG
 - MITF
- **Drug Resistance**
 - GSTP1
- **Cell Cycling**
 - CDK1
- **Adhesion/Signaling**
 - P-Cadherin (CDH3)

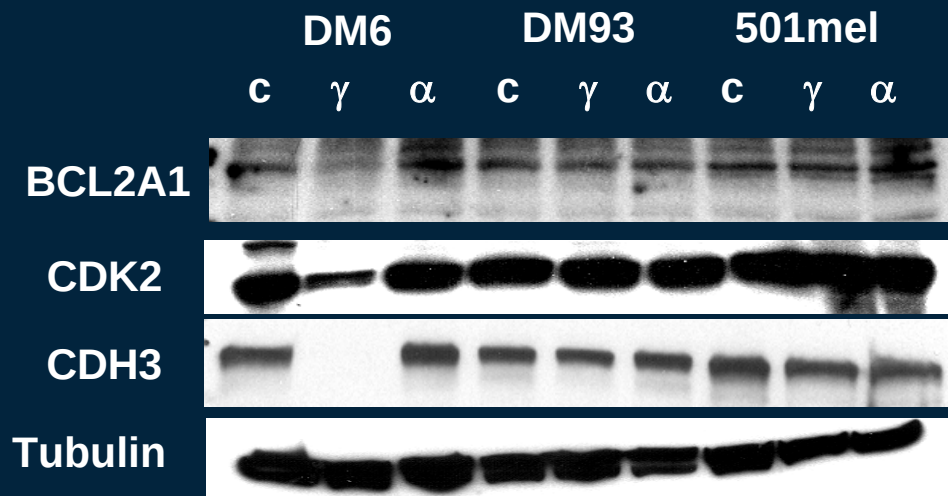
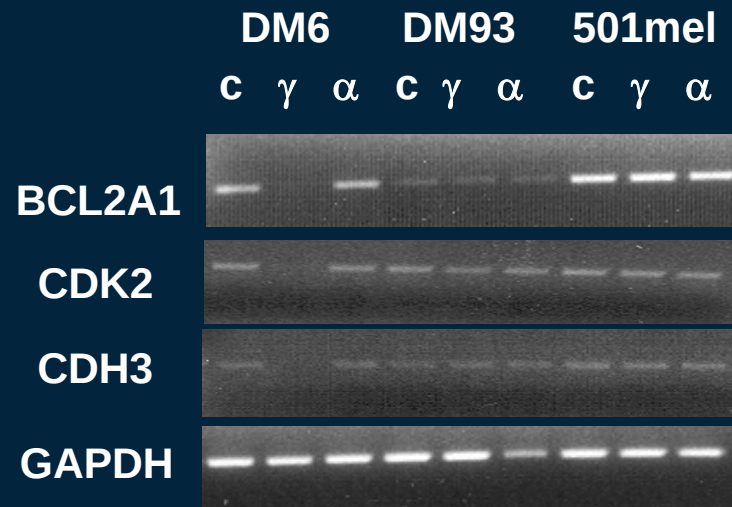
Genes Upregulated Exclusively By IFN- γ in DM6 Alone

<u>DM6</u>			<u>DM93</u>			<u>501mel</u>			GENE	
-	IFN- γ	IFN- α	-	IFN- γ	IFN- α	-	IFN- γ	IFN- α		
0.659279	12.07569	1.860501	0.543275	0.707542	0.604516	0.515542	0.353854	0.521145	DKK1	← Wnt antagonist
0.399242	6.953579	1.083402	0.512604	0.510558	0.709481	0.565811	0.88899	0.794354	ARHE	
0.537554	7.626761	1.513763	1.320762	1.664604	2.123026	2.103704	4.027425	3.041802	IL8	
0.163068	2.067221	0.59047	0.276753	0.266896	0.332907	0.256182	0.366132	0.275422	ANPEP	
0.355561	2.837478	0.793812	1.97989	2.509625	1.730379	0.376029	0.630407	0.474209	E2F5	
0.490982	3.916906	0.467567	0.409043	0.471223	0.391388	0.597968	0.4766	0.366101	UNC5H2	← Dependence receptor
2.495437	17.53875	3.115554	5.571553	8.432878	4.46949	0.839492	1.274359	0.083483	ICAM1	
2.303505	15.70121	2.921895	3.160252	3.598731	2.358484	1.704962	1.534327	1.415292	CED-6	
0.478099	2.90128	0.75179	0.471262	0.459029	0.48536	0.486225	0.176511	0.317078	MGC15563	
0.748048	4.508287	1.415425	0.851631	1.145494	1.195769	1.671579	1.658669	2.268074	NAG	
0.860162	5.018811	1.272031	1.287488	1.679722	1.734939	1.731424	1.433589	1.340598	BTG1	
0.86603	5.037277	1.179374	1.492015	1.692307	1.274837	0.699473	1.143908	0.82593	BCL6	
1.154196	6.582591	1.364566	2.63269	3.703628	2.507069	1.184337	1.368643	2.008943	TIMP3	
0.511921	2.564664	0.456347	0.072374	0.07591	0.138557	0.097764	0.103671	0.075675	ASS	
0.914276	4.567414	0.903437	2.848371	2.217516	1.402624	0.497119	0.897992	0.61098	MGC5618	
0.748829	3.649492	1.919596	0.977011	1.029039	1.01426	0.803756	1.263	1.25616	KIAA0286	
0.441606	2.138063	0.84876	0.213618	0.254055	0.337346	0.33965	0.233106	0.182904	MCL1	
0.762106	3.676233	0.988403	2.166048	2.415692	1.61117	0.621932	1.132686	0.805889	LCCP	
0.836926	3.66055	1.050624	0.597607	0.821198	0.688728	1.323524	1.710724	0.56555	LOC28614	
0.492537	2.145819	0.783085	1.244041	1.404159	1.075281	0.518355	0.762004	0.531453	BACH1	
2.31144	10.06136	3.802726	3.465735	4.575811	3.48755	1.825827	3.173086	3.236182	DLC1	← Tumor suppressor gene
2.612453	10.85297	4.342491	0.985297	0.930117	0.606571	3.934767	4.220922	3.648794	SEMA5A	
1.069218	4.414838	1.448384	0.286551	0.414868	0.415953	0.383052	0.501289	0.491296	UCHL1	
4.293284	28.31508	4.380568	3.182629	6.699052	4.51835	3.671739	3.422243	3.195018	DSCR1	← Calcineurin inhibitor
0.160793	9.036325	0.691808	0.82586	1.874211	0.812563	0.204445	0.915281	0.276427	THBS1	← Anti-angiogenic/ pro-apoptotic

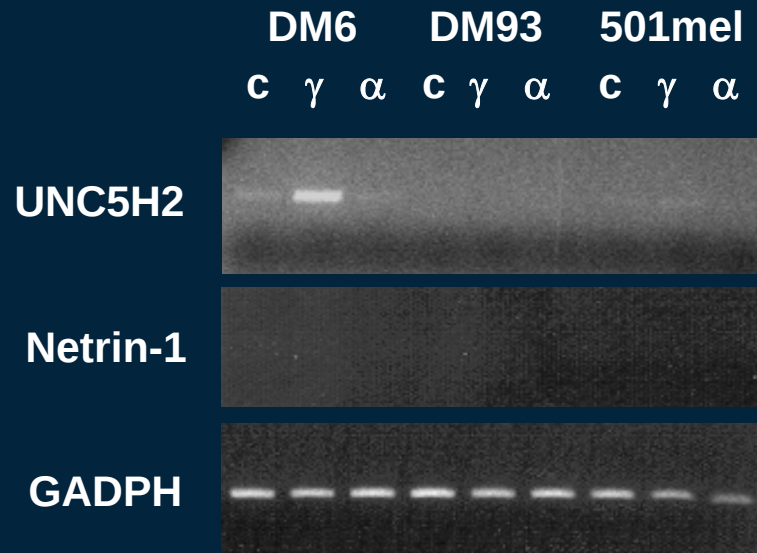
Genes of Interest Upregulated By IFN- γ in DM6

- **Wnt Signaling**
 - DKK-1
 - c-Jun
- **Apoptosis Induction**
 - UNC5H2
 - Thrombospondin-1
- **Calcium Signaling Inhibition**
 - DSCR1
- **Tumor Suppressor**
 - DLC1

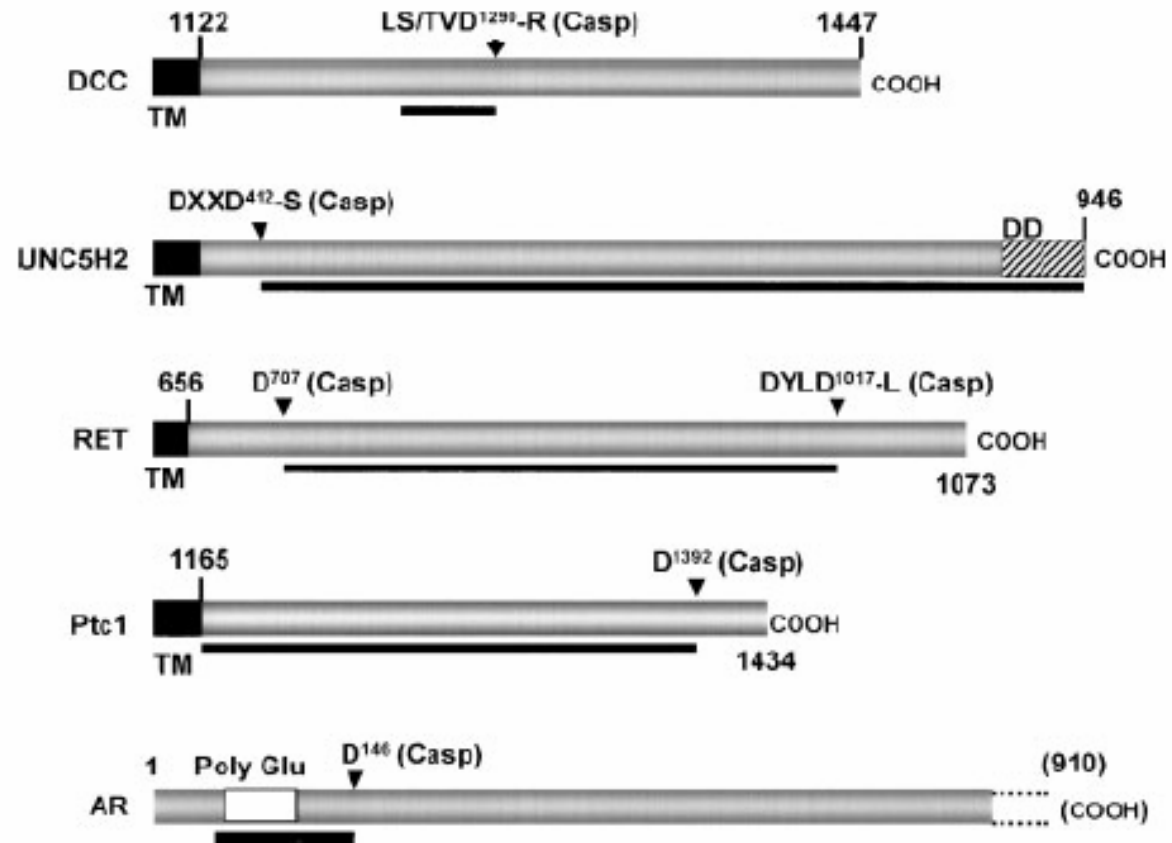
Downregulation of BCL2A1, CDK2, and CDH3 By IFN- γ in DM6



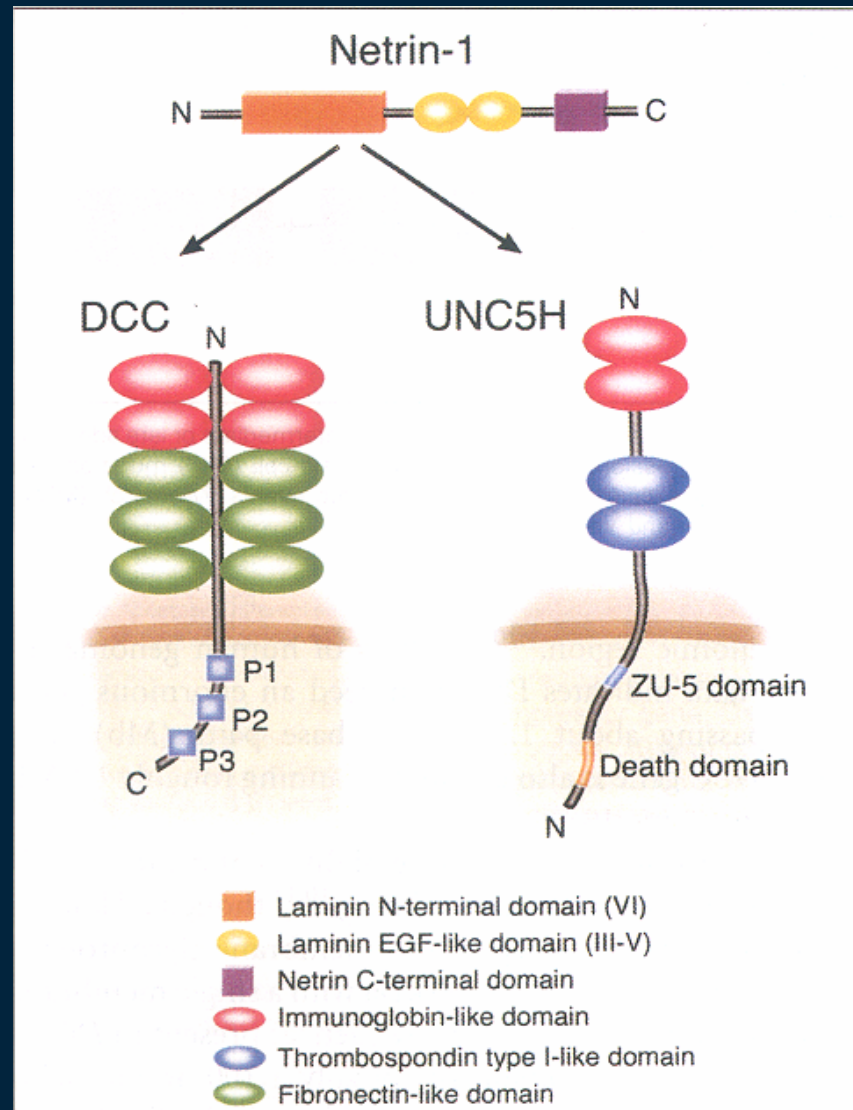
Upregulation of UNC5H2 By IFN- γ in DM6



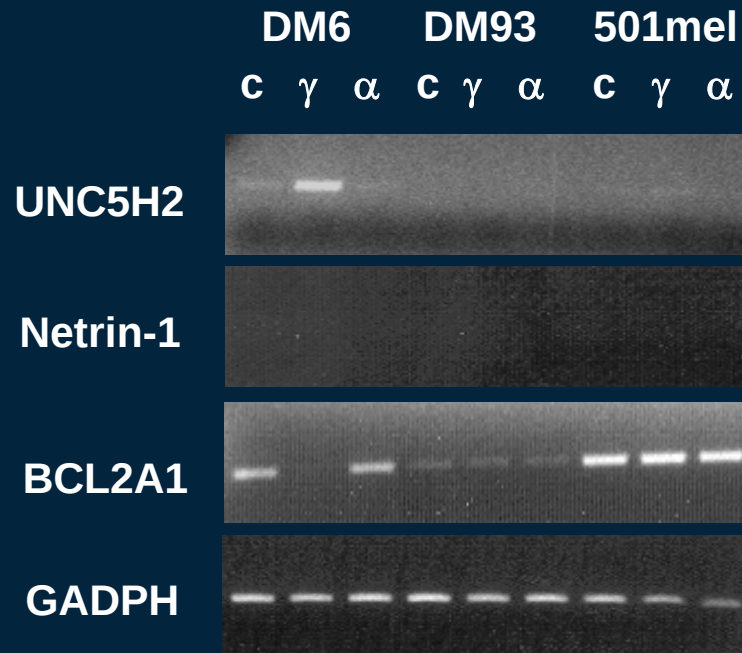
Dependence Receptors



Netrin-1 is Ligand for UNC5H and DCC Receptors



Upregulation of UNC5H2 and Downregulation of BCL2A1 By IFN- γ in DM6



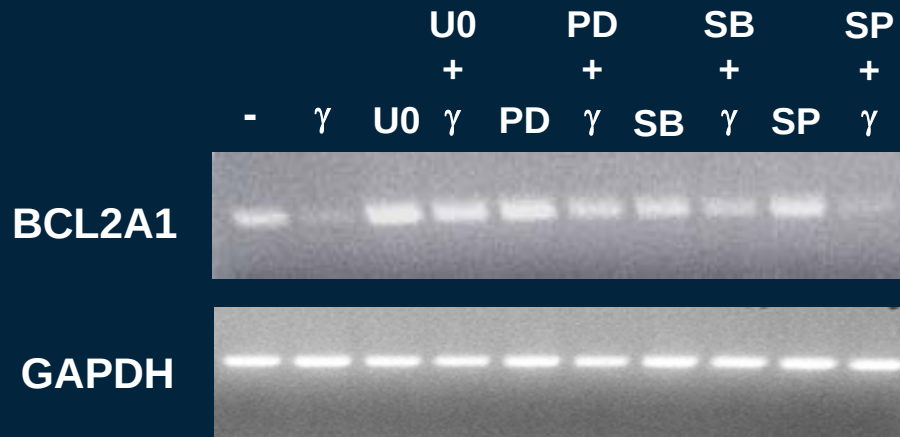
PD (MEKi) Reverses Effect of IFN- γ on Gene Expression in DM6

C	IFN- γ	IFN- γ + PD	PD				GENE
11.48431	5.172697	11.16023	12.6588	0.00212	0.369761	0.656487	TNFRSF14
3.002219	0.806102	2.962136	4.791052	0.015824	0.013582	0.851589	PIR
5.624756	2.061474	4.34536	4.628681	0.000854	0.025815	0.012916	GPRC5B
2.631482	0.918191	1.805041	2.939648	0.000967	0.077959	0.002656	RAP2B
4.38136	2.087006	4.972922	6.0911	0.004324	0.019476	0.194859	RAGD
5.224237	2.528697	9.295827	4.795968	0.016836	0.447223	0.001347	CA14
8.221466	1.183709	5.212186	8.979258	0.008056	0.3921	0.040478	BCL2A1
3.001584	1.038414	4.498802	5.18968	0.001304	0.00023	0.006355	GSTP1
10.17658	1.830574	17.48152	18.96298	0.00461	0.00064	0.019812	MLANA
3.144808	1.359729	3.628799	6.445852	0.001248	4.43E-05	0.208991	RAB38
4.480912	1.295735	9.934932	12.77903	0.002643	6.05E-05	6.04E-05	SLUG
2.296821	0.999177	3.826646	4.669832	0.00082	0.000121	0.164842	EDNRB
3.934885	1.628624	5.293474	11.03099	0.005022	0.012231	0.182588	MGC31963
4.046701	0.824866	3.105258	5.630735	3.48E-06	0.033881	0.051033	
5.283698	2.236524	7.067819	12.06758	0.000124	0.0131	0.119767	ASAH
5.960505	2.098791	6.576778	10.37931	0.005587	0.000552	0.198105	AP1S2
5.616617	2.151537	4.403805	3.93623	0.019016	0.108995	0.218643	CAPN3
3.217655	0.592665	2.584436	4.996113	0.000123	0.052774	0.002743	CDK2
9.014619	2.798744	5.033279	12.47508	0.00986	0.021768	0.016532	GPR56
3.287959	0.805048	3.0882	3.592157	0.02265	0.640139	0.67205	
6.360403	0.644942	2.601155	9.883589	0.004946	0.003529	0.005548	CDH3
2.394306	0.832868	1.86821	4.280225	0.00165	0.06665	0.063835	TYR
6.07324	1.858976	4.023941	5.325568	3.80E-07	0.229121	0.010317	LGALS3

PD (MEKi) Reverses Effect of IFN- γ on Gene Expression in DM6

C	IFN- γ +			GENE			
	IFN- γ	PD	PD				
0.36945	2.631749	0.628069	0.402045	0.00241	0.29351	0.001566	SOD2
0.100121	7.581813	0.926967	0.202982	0.001791	0.082735	0.000181	SCYA2
1.666318	4.381268	1.249507	1.165373	0.01057	0.045737	0.083964	PLAT
1.658291	9.043001	3.874699	1.745538	0.003444	0.645853	0.055977	EPB49
1.601459	13.65161	1.24428	0.71322	3.25E-07	0.004187	0.060191	TIMP3
0.841032	6.713785	1.423623	0.468326	0.00027	0.023102	0.003614	FER1L3
3.617299	43.27787	9.089277	1.503985	7.30E-06	0.06238	0.004259	DSCR1
1.326233	7.752615	1.956823	0.394813	0.014107	0.001058	0.083907	UNC5H2
0.649866	2.532485	0.577874	0.488223	0.00017	0.067959	0.286885	BACH1
1.928922	6.803482	2.509298	1.566651	0.000942	0.000993	0.000487	MAP1A/1B
0.30604	4.07581	1.104095	0.312081	0.001151	0.968502	0.002258	DEPP
0.573144	2.231067	0.887107	0.599646	0.002101	0.151798	0.042343	CRHSP-24
0.461188	2.203343	0.751107	0.583123	5.77E-06	0.253815	0.008608	HDGF
0.144255	4.089554	0.620588	0.249376	0.003199	0.133498	0.029901	THBS1
1.683359	5.047885	0.5487	0.503951	0.000809	1.65E-05	2.78E-05	MGC17528
1.133405	2.962733	1.416051	0.934861	0.015259	0.144404	0.123853	ARF4L
1.632888	3.183415	1.710761	0.88042	0.005016	0.010037	0.557172	DUSP6
0.456762	3.618618	0.956834	0.692033	9.30E-06	0.005636	0.000532	MGC5618
0.795023	5.099768	1.840154	0.950131	0.012849	0.195325	0.11444	CSF1
1.644852	2.833254	1.185778	0.125269	0.00341	0.007097	0.061921	ASS
0.892512	5.635059	1.915934	0.847702	0.004461	0.642769	0.027841	BTG1
4.026003	12.05308	6.133041	2.159208	0.000273	0.003107	0.049972	PPP1R15A
1.124344	6.438722	2.841256	0.703281	0.002201	0.000203	0.002725	UCHL1
1.08309	3.381357	1.857783	0.737733	0.006809	0.000181	0.050079	PKD1
1.273506	6.708882	1.199133	0.524732	0.003828	0.003049	0.58974	VEGF
1.415251	6.64125	1.170433	0.553651	0.007731	9.20E-05	0.060109	LOC28614
2.94694	5.974664	2.566798	2.376168	0.012266	0.007492	0.076967	TNFRSF10
0.716296	6.989198	0.710716	0.473055	0.007134	0.097602	0.909671	DDK1

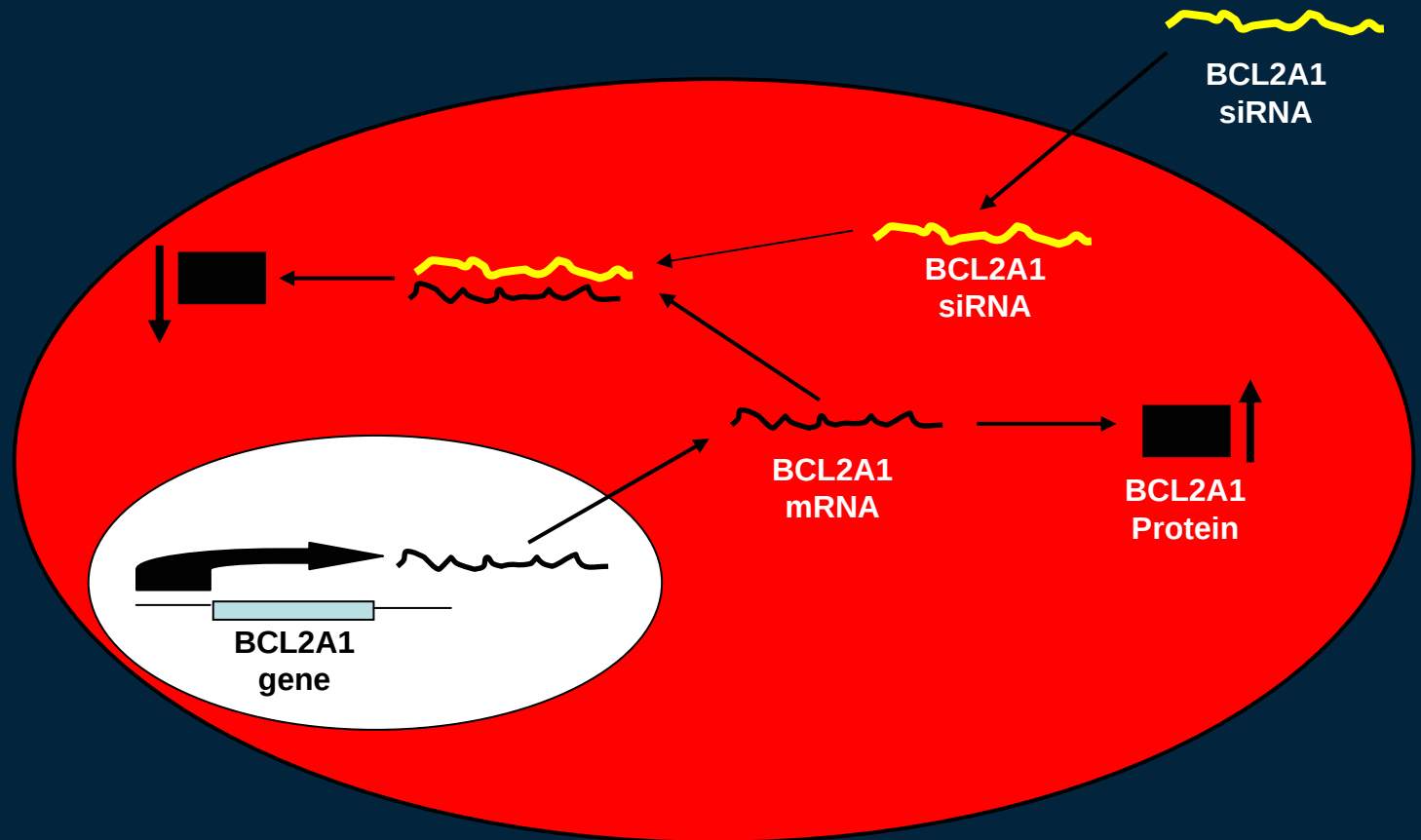
MEK or p38 Inhibition Blocks Downregulation of BCL2A1 By IFN- γ in DM6



MAPKs and Modulation of Gene Expression By IFN- γ in Melanoma

- There is a short list of genes modulated only by IFN- γ in the one cell line (DM6) sensitive to the pro-apoptotic/antiproliferative effects of IFN- γ
- The modulation of 50-75% of those genes by IFN- γ is MEK1-dependent, with a significant proportion also p38-dependent
- Since PD (MEK1i) and SB (p38i) block the antimelanoma effect of IFN- γ , some or all of these genes whose modulation by IFN- γ is controlled by PD and SB are likely central to this effect

Gene Knockout Via RNA Interference



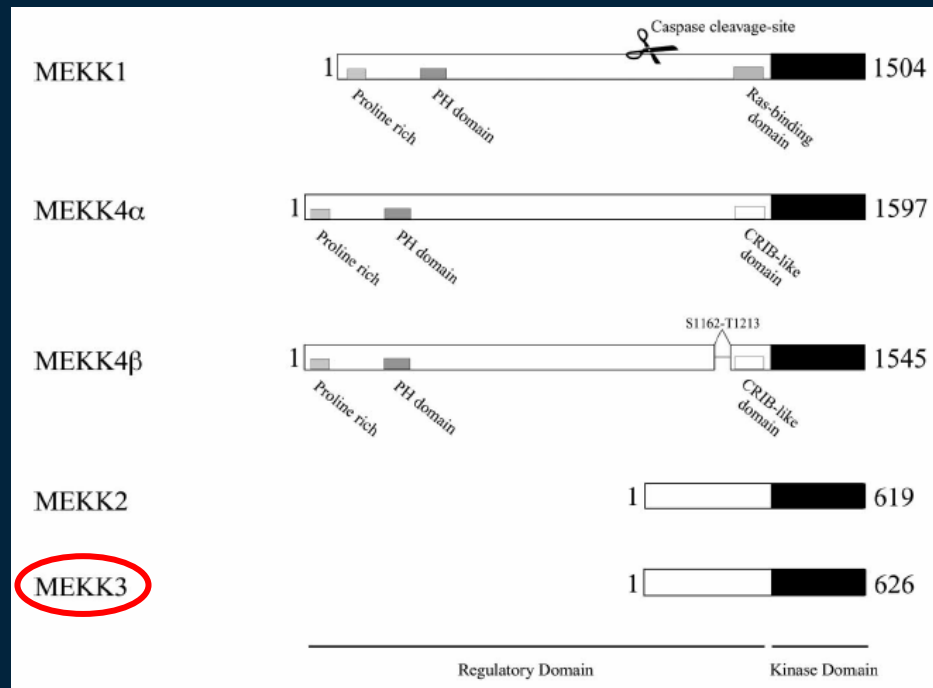
DM6 Melanoma

Direct Effects of Interferons on Melanoma: Questions

- Which signaling pathways mediate the direct pro-apoptotic and antiproliferative effects of IFN?
- Do interferons alter the expression of certain key genes to achieve these effects?
- What is the molecular basis for resistance of melanoma to these effects of interferons, and can this resistance be overcome?

MEKK3: Role in Melanoma Resistance to IFN- γ ?

- Serine-threonine kinase
- Conserved **kinase domain**
- Small **regulatory domain**
- Activation modified through interaction with:
 - **BRCA1**
 - **TRAF7**



A vertical strip on the left side of the slide shows a microscopic image of biological cells, possibly red blood cells, in a reddish hue.

Acknowledgements

- Zhiqing Huang, MD, PhD
- Catherine Sciambi, BS