

Tasquinimod Suppresses Tumor Cell Growth and Bone Resorption by Targeting Immunosuppressive Myeloid Cells and Inhibiting c-MYC Expression in Multiple Myeloma

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Supplementary Material and Methods

Reagents

Recombinant mouse IL-10 (#575804, biolegend) was used at a concentration of 100 ng/mL for the T cell suppression assay. The HDAC inhibitor panobinostat (LBH589, Novartis Pharmaceuticals) was purchased from Selleckchem (East Hanover, NJ) and prepared as a 10 mM stock solution in dimethylsulfoxide and stored at -80°C.

Gene expression data

The online webtool GenomicScape (<http://genomicscape.com/microarray/survival.php>) was consulted for the microarray data of the TT2 cohort (accession number: GSE204225, <http://www.ncbi.nlm.nih.gov/geo/>). Gene expression of HDAC4 was analyzed in bone marrow plasma cells (BMPCs) of healthy individuals (n = 22) and in patient samples at different MM substages including monoclonal gammopathy of undetermined clinical significance (MGUS) (n = 44), smoldering multiple myeloma (SMM) (n = 12) and CD138⁺ cells of newly diagnosed MM patients (n = 345) and primary MM cell lines (n = 23). Gene expression data were normalized with the MAS5 algorithm.

Cell culture

MM cell lines were seeded at a concentration of 2.5×10^5 cells/mL with different concentrations of tasquinimod (10, 25 μ M) for 3 days and 5 days in 1% FCS RPMI medium. Apoptosis was measured by flow cytometry using the Annexin-V/7-AAD staining. MM cell lines were seeded at a concentration of 5.0×10^5 cells/mL with different concentrations of panobinostat (0.5, 1 μ M) for 6 h and 24 h.

Cell viability

CD11b⁺ cells were sorted from 5TGM1 model and 5T33MM model by CD11b microbeads and seeded in a white, opaque 96-well plate. The following day, cells were treated with tasquinimod and cell viability was measured at 24 h post-treatment using the CellTiter-Glo[®] Luminescent Cell Viability Assay (Promega, G7573)

according to manufacturer's instructions. Microplate luminescence was measured using a CLARIOstar (BMG Labtech) plate reader. All data were normalized to non-treated controls.

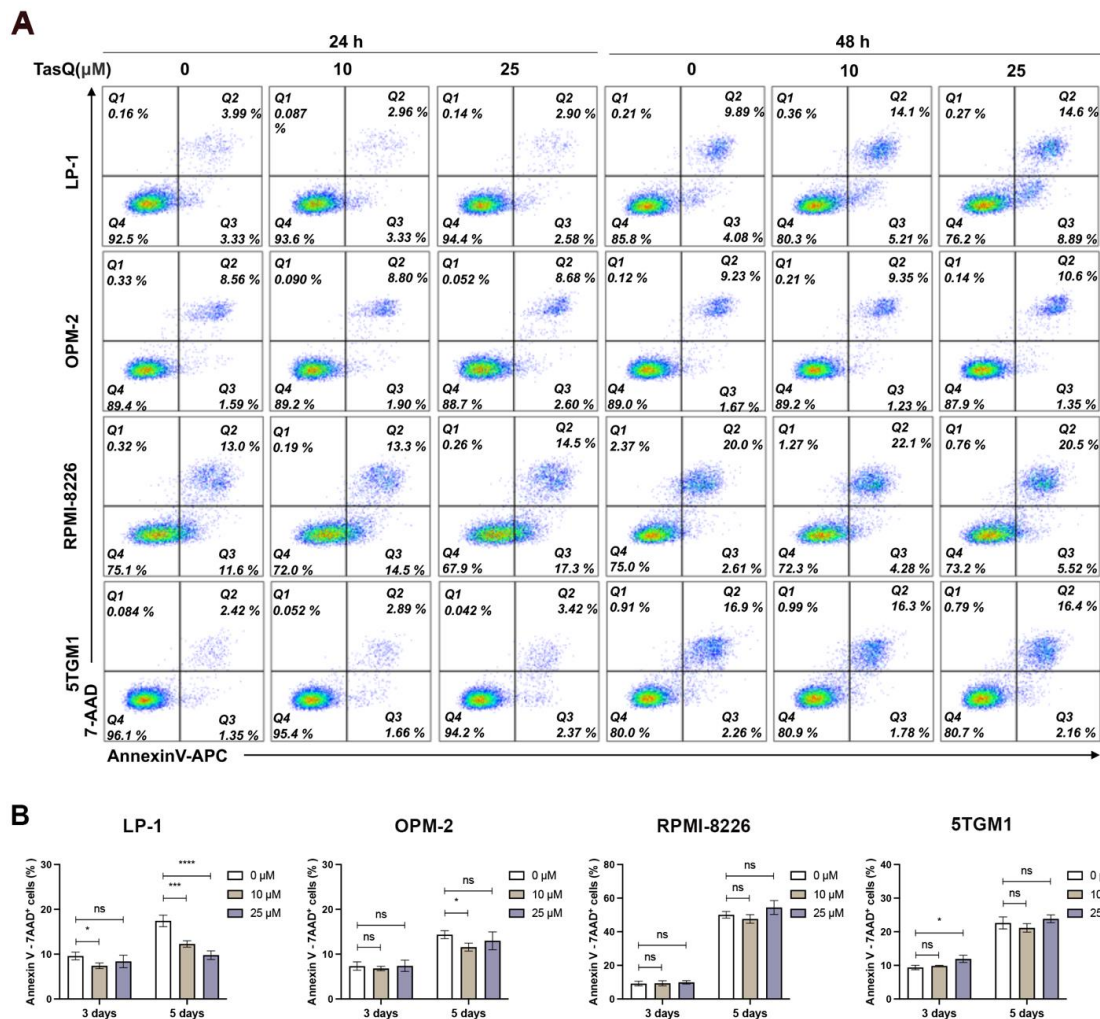
Supplementary Table 1. Mouse primer sequences of genes evaluated by real-time quantitative PCR

Gene		Primer sequence 5'-3'
<i>Abl</i>	Forward	CCG TGG GTG CCA CTA TAT TT
	Reverse	GGG CAC AGT GGT GAA CTA TT
<i>Arg1</i>	Forward	ACA GCA AAG CAG ACA GAA CTA
	Reverse	GAA AGG AAC TGC TGG GAT ACA
<i>Fizz1</i>	Forward	CCC AGT GAA TAC TGA TGA GAC C
	Reverse	GGA GGG ATA GTT AGC TGG ATT G
<i>Mrc1</i>	Forward	GGA ATC AAG GGC ACA GAG TTA
	Reverse	TTC CAT CTG CTC CAC AAT CC
<i>Nos2</i>	Forward	GGA ATC TTG GAG CGA GTT GT
	Reverse	CCT CTT GTC TTT GAC CCA GTA G
<i>Stab1</i>	Forward	ACG GGA AAC TGC TTG ATG TC
	Reverse	ACT CAG CGT CAT GTT GTC CA
<i>Tnfa</i>	Forward	CTA CCT TGT TGC CTC CTC TTT
	Reverse	GAG CAG AGG TTC AGT GAT GTA G
<i>IL10</i>	Forward	TTG AAT TCC CTG GGT GAG AAG
	Reverse	TCC ACT GCC TTG CTC TTA TTT
<i>c-Myc</i>	Forward	GCT GTT TGA AGG CTG GAT TTC
	Reverse	GAG TCG TAG TCG AGG TCA TAG T

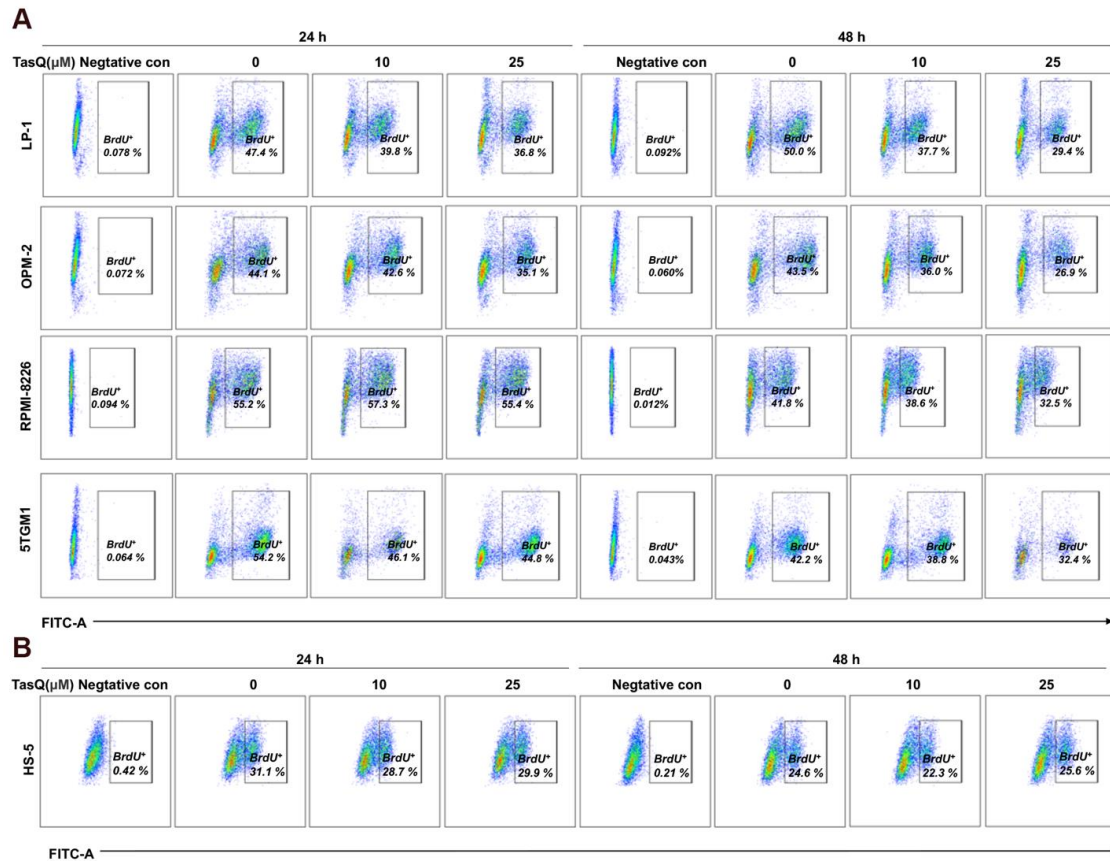
Supplementary Table 2. Differentially expressed genes in LP-1 cells treated with tasquinimod. Using RNA sequencing, we identified a list of upregulated and downregulated genes in LP-1 cells treated with tasquinimod (25 µM) for 6 h and 24 h ($\text{padj} \leq 0.05$ & $|\log_2\text{FoldChange}| \geq 1$) (n=3).

Up regulated genes in TasQ treated LP-1 (6 h)													
Ensembl ID	Gene symbol	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Cnt1	Cnt2	Cnt3	Tas1	Tas2	Tas3
ENSG00000143153	ATP1B1	103.621196	1.018170392	0.19703	5.16752	2.37E-07	4.03E-06	69.38256	72.4779	64.0296	143.197	118.881	153.76
ENSG00000125266	EFNB2	86.3883412	1.035125581	0.21339	4.85077	1.23E-06	1.79E-05	64.20476	55.4909	50.5022	127.611	113.927	106.594
ENSG00000107074	HFE	104.822284	1.08717393	0.21785	4.99036	6.03E-07	9.48E-06	67.31144	57.7558	75.7533	106.18	143.647	178.286
ENSG00000154309	DISP1	64.3172858	1.102929222	0.24203	4.55691	5.19E-06	6.59E-05	41.42243	39.6364	41.4839	91.5681	81.2351	90.5578
ENSG0000050405	LIMA1	86.8832765	1.174777021	0.2192	5.35946	8.35E-08	1.55E-06	43.49355	50.961	64.9314	118.844	131.759	111.311
ENSG00000076641	PAG1	354.290158	1.307707212	0.11141	11.7373	8.20E-32	1.80E-29	187.4365	220.831	203.812	513.366	521.094	479.202
ENSG00000196083	IL1RAP	83.2898715	1.415285922	0.222	6.37525	1.83E-10	5.31E-09	40.38687	44.1662	51.404	116.895	112.937	133.95
ENSG00000164741	DLC1	74.4918423	1.448455883	0.23361	6.20023	5.64E-10	1.50E-08	39.35131	41.9013	38.7785	104.232	100.058	122.63
ENSG00000197019	SERTAD1	59.4743365	1.608127034	0.26411	6.08879	1.14E-09	2.84E-08	25.88902	30.5766	31.5639	95.4646	94.1139	79.2381
Down regulated genes in TasQ treated LP-1 (6 h)													
Ensembl ID	Gene symbol	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Cnt1	Cnt2	Cnt3	Tas1	Tas2	Tas3
ENSG00000169245	CXCL10	1555.72273	-1.726671085	0.06807	-25.364	6.22E-142	7.38E-138	2396.287	2307.97	2462.88	782.225	734.088	650.884
ENSG00000168329	CX3CR1	80.4909195	-1.690891097	0.22995	-7.3534	1.93E-13	8.30E-12	125.3028	114.379	128.961	30.198	42.5989	41.5057
ENSG00000168334	XIRP1	465.496837	-1.657655626	0.10225	-16.212	4.14E-59	4.15E-56	716.608	756.488	648.412	225.998	221.911	223.555
ENSG00000183625	CCR3	219.756282	-1.597770032	0.13972	-11.436	2.77E-30	5.39E-28	303.4193	343.138	344.497	110.076	107.983	109.424
ENSG00000076356	PLXNA2	514.954525	-1.388409651	0.09444	-14.701	6.35E-49	4.19E-46	794.275	738.369	703.423	289.316	281.351	282.993
ENSG00000128917	DLL4	245.673963	-1.350305566	0.13494	-10.007	1.43E-23	1.84E-21	385.2286	349.932	323.755	151.964	137.703	125.46
ENSG00000169085	VXN	98.0317411	-1.333117655	0.20855	-6.3922	1.63E-10	4.77E-09	128.4095	157.413	135.274	63.3183	59.4403	44.3356
ENSG00000223863	LINC01805	153.07184	-1.319443666	0.16739	-7.8823	3.21E-15	1.77E-13	225.7522	210.639	219.143	105.206	82.2258	75.4648
ENSG00000232591	LINC02642	56.6622161	-1.20975926	0.26068	-4.6409	3.47E-06	4.56E-05	86.9871	69.0805	81.1642	36.0427	33.6829	33.0159
ENSG00000221869	CEBPD	51.026088	-1.192533363	0.27693	-4.3063	1.66E-05	0.0001819	82.84485	67.948	62.2259	32.1462	32.6922	28.2993
ENSG00000165970	SLC6A5	74.8623777	-1.190963311	0.22891	-5.2027	1.96E-07	3.42E-06	97.3427	107.584	107.317	50.6547	48.5429	37.7324
ENSG00000107984	DKK1	49.7665669	-1.185073346	0.29999	-3.9504	7.80E-05	0.0007194	59.02696	82.6701	65.8332	42.8616	21.7948	26.4127
ENSG00000100368	CSF2RB	130.748422	-1.145159047	0.1747	-6.5549	5.57E-11	1.71E-09	190.5432	171.003	178.561	92.5422	78.2631	73.5782
ENSG00000053918	KCNQ1	54.4190729	-1.119346433	0.26799	-4.1768	2.96E-05	0.0003028	83.88041	70.213	69.4405	33.1204	40.6176	29.2426
ENSG00000178965	ERIC3	214.922947	-1.117761094	0.13701	-8.1583	3.40E-16	2.05E-14	280.6369	294.441	307.522	148.067	126.806	132.063
ENSG00000184828	ZBTB7C	67.6005665	-1.112931413	0.24167	-4.6052	4.12E-06	5.36E-05	88.02266	101.922	87.477	37.991	51.515	38.6757
ENSG00000181291	TMEM132E	277.330627	-1.11026495	0.12427	-8.9342	4.10E-19	3.40E-17	382.1219	412.218	343.595	181.188	169.405	175.456
ENSG00000163823	CCR1	1203.27605	-1.109650045	0.06845	-16.211	4.19E-59	4.15E-56	1699.355	1688.51	1546.63	801.708	742.013	741.442
ENSG00000053108	FSTL4	177.824507	-1.095425979	0.15829	-6.9204	4.50E-12	1.63E-10	201.9343	270.66	254.315	120.792	106.993	112.254
ENSG00000127831	VIL1	222.089259	-1.07598418	0.14052	-7.6574	1.90E-14	8.96E-13	289.957	274.057	339.086	131.507	154.545	143.383
ENSG00000185862	EVI2B	369.016225	-1.030782678	0.10822	-9.5251	1.65E-21	1.79E-19	478.429	483.563	523.96	263.989	237.761	226.395

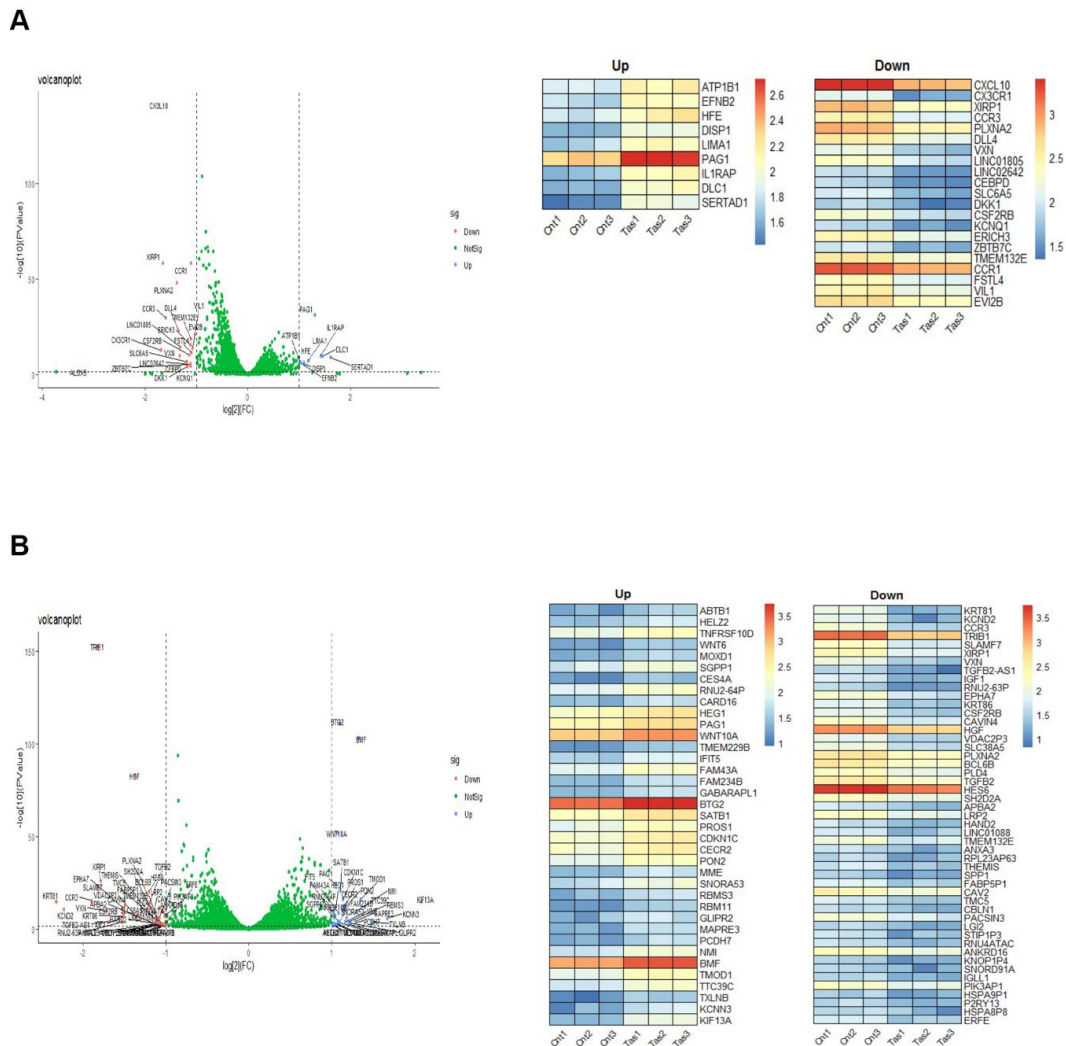
Up regulated genes in TasQ treated LP-1 (24 h)													
Ensembl ID	Gene symbol	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	Cnt1	Cnt2	Cnt3	Tas1	Tas2	Tas3
ENSG00000139112	GABARAPL1	38.3711148	1.094614799	0.31661	3.45729	0.0005456	0.0039602	24.33034	24.4594	24.7579	45.0197	58.41	53.2493
ENSG00000084444	FAM234B	38.3419731	1.087872499	0.31504	3.45311	0.0005542	0.0040157	26.54219	25.4001	21.7869	48.3139	54.7594	53.2493
ENSG00000144642	RBMS3	38.3906879	1.200500052	0.32691	3.67228	0.0002404	0.0019633	17.6948	29.1631	22.7772	46.1178	52.9341	61.6571
ENSG00000084764	MAPRE3	35.7753684	1.285215457	0.33334	3.85558	0.0001155	0.0010567	22.11849	24.4594	15.845	51.608	51.1088	49.5125
ENSG00000204397	CARD16	38.9500164	1.047412393	0.32858	3.18768	0.0014342	0.0088204	25.43627	31.9854	18.816	45.0197	64.7986	47.6441
ENSG00000130589	HELZ2	44.7308589	1.02031668	0.30959	3.29574	0.0009816	0.0064715	29.85997	24.4594	34.661	42.8236	71.1872	65.3939
ENSG00000143603	KCNC3	39.9964207	1.667010993	0.35299	4.7225	2.33E-06	3.40E-05	11.05925	28.2224	17.8257	46.1178	63.886	72.8675
ENSG00000122694	GLIPR2	40.7785087	1.212019906	0.34992	3.46372	0.0005328	0.0038849	22.11849	15.0519	36.6416	60.3923	69.3619	41.1047
ENSG00000152778	IFIT5	56.0192944	1.073961253	0.26541	4.0465	5.20E-05	0.0005323	42.02514	37.6298	28.7191	79.059	73.0126	75.6701
ENSG00000185272	RBM11	24.9968283	1.209443943	0.39208	3.0847	0.0020376	0.011871	15.48295	15.9927	13.8644	30.7452	35.9936	38.3021
ENSG00000198133	TMEM229B	22.896663	1.072243037	0.40585	2.64194	0.0082432	0.0370817	14.37702	14.1112	15.845	27.4511	31.0303	34.5653
ENSG00000115596	WNT6	24.9742134	1.024565383	0.3904	2.62439	0.0086804	0.0387148	17.6948	18.8149	12.8741	34.0393	35.9936	30.8286
ENSG00000164440	TXLNB	23.1095368	1.486864327	0.42398	3.50694	0.0004533	0.0033764	12.16517	8.46671	15.845	38.4315	30.1177	33.6311
ENSG00000107931	MOXD1	31.486598	1.027573766	0.35121	2.92581	0.0034357	0.018275	18.80072	22.5779	20.7966	37.3334	51.1088	38.3021
ENSG00000114626	ABTB1	27.6180976	1.009163945	0.38629	2.61246	0.0089892	0.0398996	17.6948	24.4594	12.8741	28.5491	42.8949	39.2363
ENSG00000169851	PCDH7	33.3543784	1.29059107	0.35912	3.59377	0.0003259	0.0025422	18.80072	23.5186	15.845	34.0393	58.41	49.5125
ENSG00000123609	NMI	98.9780823	1.302167082	0.20248	6.43094	1.27E-10	4.68E-09	54.19031	51.741	65.3608	142.745	136.899	142.932
ENSG00000126821	SGPP1	98.7357042	1.030442916	0.21124	4.87814	1.07E-06	1.71E-05	50.87254	83.7264	59.4189	130.667	135.073	132.656
ENSG00000212443	SNORA53	86.940508	1.182301894	0.24177	4.89028	1.01E-06	1.63E-05	40.91921	46.0965	72.293	115.294	101.305	145.735
ENSG00000168234	TTC39C	108.035083	1.397721754	0.19968	6.99969	2.57E-12	1.26E-10	60.82586	55.504	62.3898	135.059	158.802	175.629
ENSG00000137177	KIF13A	65.0820315	2.083882597	0.26447	7.87937	3.29E-15	2.60E-13	24.33034	23.5186	26.7385	98.8238	104.043	113.038
ENSG00000173530	TNFRSF10D	158.126012	1.023609848	0.16072	6.36871	1.91E-10	6.86E-09	103.9569	111.949	97.0508	196.55	229.99	209.26
ENSG00000184500	PROS1	155.449464	1.13167493	0.17774	6.36691	1.93E-10	6.92E-09	79.62658	111.008	101.012	209.726	249.155	182.169
ENSG00000136842	TMOD1	164.547344	1.347966482	0.16243	8.29886	1.05E-16	1.01E-14	94.0036	85.6079	99.0315	238.275	257.369	212.997
ENSG00000105354	PON2	149.826755	1.161267053	0.16487	7.04344	1.88E-12	9.37E-11	96.21545	90.3116	91.109	225.099	192.517	203.655
ENSG00000185112	FAM43A	128.07146	1.082939209	0.18293	5.91984	3.22E-09	8.95E-08	84.05028	82.7856	79.2252	199.844	148.763	173.761
ENSG00000223247	RNU2-64P	123.411963	1.043965427	0.19258	5.42084	5.93E-08	1.27E-06	76.30881	76.2004	89.1283	171.295	134.161	193.379
ENSG00000182568	SATB1	326.140889	1.1039324	0.11648	9.47759	2.60E-21	4.41E-19	211.2316	210.727	200.044	405.178	469.106	466.56
ENSG00000173706	HEG1	315.566233	1.048705617	0.14871	7.05181	1.77E-12	8.92E-11	162.5709	214.49	239.656	342.589	439.901	494.191
ENSG00000129757	CDKN1C	247.077386	1.13441816	0.13693	8.28465	1.18E-16	1.12E-14	171.4183	142.995	150.528	315.138	322.168	380.219
ENSG00000099954	CECR2	232.514568	1.14131831	0.15895	7.18032	6.95E-13	3.71E-11	190.2191	130.764	115.867	288.785	332.207	337.246
ENSG00000166641	PAG1	414.236923	1.053885463	0.10297	10.235	1.38E-24	3.05E-22	266.5279	270.935	270.356	557.805	527.516	592.282
ENSG00000104081	BMF	2184.0285	1.331990767	0.0615	21.6597	4.92E-104	2.28E-100	1143.526	1314.22	1265.62	3000.95	3197.04	3182.81
ENSG00000135925	WNT10A	1146.39434	1.065875055	0.06988	15.2524	1.59E-52	2.75E-49	757.5584	713.085	754.62	1469.18	1615.4	1568.52
ENSG00000159388	BTG2	3761.03942	1.101356129	0.049	22.476	1.73E-112	4.95E-108	2490.542	2294.48	2391.61	5117.97	5219.48	5052.15
Down regulated genes in TasQ treated LP-1 (24 h)													
Ensembl ID	Gene symbol	baseMean	log3FoldChange	lfcSE	stat	pvalue	padj	Cnt1	Cnt2	Cnt3	Tas1	Tas2	Tas3
ENSG00000213262	VDAC2P3	57.7394309	-1.354138043	0.26711	-5.0696	3.99E-07	7.16E-06	88.47398	68.6744	92.0993	30.7452	34.681	31.7627
ENSG00000100368	CSF2RB	57.1941831	-1.508054408	0.26866	-5.6132	1.99E-08	4.75E-07	89.5799	81.8449	82.1961	35.1373	30.1177	24.2892
ENSG00000170442	KRT86	48.7800833	-1.516178725	0.29138	-5.2034	1.96E-07	3.81E-06	71.88511	63.9707	81.2058	23.0589	29.205	23.355
ENSG00000184408	KCND2	46.7007639	-2.228640509	0.33568	-6.6391	3.16E-11	1.27E-09	86.26213	61.1485	83.1864	20.8628	9.12657	19.6182
ENSG00000169085	VXN	63.9637778	-1.712894755	0.26951	-6.3556	2.08E-10	7.39E-09	116.1221	95.9561	82.1961	29.6471	36.5063	23.355
ENSG00000205426	KRT81	55.2654673	-2.318266133	0.29319	-7.907	2.64E-15	2.10E-13	95.10953	91.2523	90.1186	15.3726	18.2531	21.4866
ENSG0000017483	SLC38A5	71.2630185	-1.339466175	0.23771	-5.6348	1.75E-08	4.23E-07	98.4273	95.0153	112.896	42.8326	41.9622	36.4337
ENSG00000165912	PAC1N3	97.1483993	-1.077000373	0.21803	-4.9398	7.82E-07	1.30E-05	129.3932	133.586	132.702	59.2943	82.1391	45.7757
ENSG00000181291	TMEM132E	92.1484108	-1.108695153	0.22173	-5.0003	5.72E-07	9.94E-06	107.2747	123.238	147.557	47.2158	55.6721	71.9333
ENSG00000135333	EPHA7	93.3607836	-1.516470409	0.21986	-6.8974	5.30E-12	2.48E-10	152.6176	115.712	146.567	54.9021	50.1961	40.1705
ENSG00000183625	CCR3	80.0337247	-1.897577666	0.23443	-8.0943	5.76E-16	5.13E-14	139.3465	120.415	118.838	35.1373	33.7683	32.6969
ENSG00000181631	P2RY13	28.4155885	-1.010563421	0.36929	-2.7365	0.0062092	0.0295291	32.07182	47.0373	34.661	19.7648	17.3405	19.6182
ENSG00000153012	LG12	27.4343776	-1.067358625	0.38008	-2.8082	0.0049812	0.0247027	33.17774	44.2151	33.6707	23.0589	14.6025	15.8814
ENSG00000232480	TGFBI-AS1	24.7186442	-1.682805052	0.42699	-3.9411	8.11E-05	0.0007836	33.17774	47.0373	32.6804	14.2745	14.6025	6.53939
ENSG00000243721	RPL23AP63	28.2751081	-1.101411561	0.38623	-2.8517	0.0043484	0.0221231	32.07182	44.2151	39.6126	12.0785	15.5152	26.1576
ENSG00000128322	IGLL1	26.0535864	-1.023050968	0.38286	-2.6721	0.0075376	0.0344589	33.17774	31.0446	40.6029	16.4706	20.0785	14.9472
ENSG00000232422	KNOP1P4	23.5731254	-1.035439331	0.40485	-2.5576	0.01054	0.0453477	36.49552	29.1631	29.7094	10.9804	17.3405	17.7498
ENSG00000104218	CSPP1	1963.22444	-0.15807164	0.06137	-2.5755	0.0100087	0.0435203	2120.058	2185.35	1906.36	1868.87	1850.87	1847.84
ENSG00000227274	RNU2-63P	23.8606376	-1.522495048	0.41287	-3.6876	0.0002264	0.0018677	40.91921	36.6891	28.7191	10.9804	12.7772	13.0788
ENSG00000212163	SNORD91A	25.6850497	-1.033595069	0.41013	-2.5202	0.0117298	0.0493652	39.81329	36.6891	26.7385	23.0589	9.12657	18.684
ENSG00000264229	RNU4ATAC	32.9343923	-1.039407002	0.34296	-3.0307	0.0024398	0.0137981	50.87254	40.4521	41.5932	23.0589	17.3405	24.2892
ENSG00000164107	HAND2	32.8757747	-1.134042773	0.34761	-3.2624	0.0011047	0.0071171	56.40216	38.5706	40.6029	21.9608	19.1658	20.5524
ENSG00000102924	CBLN1	29.7085901	-1.077588891	0.36622	-2.9425	0.0032561	0.0174747	44.23699	33.8668	42.5835	25.255	16.4278	15.8814
ENSG00000138772	ANXA3	32.5801694	-1.104795578	0.35916	-3.076	0.0020978	0.0121765	48.66069	49.8595	34.661	25.255	13.6899	23.355
ENSG00000103534	TMC5	41.6362064	-1.081108857	0.30544	-3.5395	0.0004009	0.0030442	66.35548	48.9188	54.4673	27.4511	26.467	26.1576
ENSG00000174727	IGF1	3.78E+01	-1.559730689	0.33296	-4.6844	2.808E-06	4.032E-05	67.46141	50.8002708	51.4963691	17.5688749	17.340481	22.4207643
ENSG0000034053	APBA2	36.7593474	-1.16209464	0.33219	-3.4983	0.0004683	0.0034732	58.61401	47.978	45.5545	29.6471	20.0785	18.684
ENSG00000249307	LINC01088	38.386322	-1.125207759	0.3356	-3.3528	0.0008	0.0054284	54.19031	54.5633	49.5157	16.4706	20.0785	35.4995
ENSG00000225536	STIP1P3	33.3462525	-1.053589342	0.36262	-2.9055	0.0036667	0.0192542	49.76661	43.2743	42.5835	10.9804	30.1177	23.355
ENSG00000130775	THEMIS2	254											



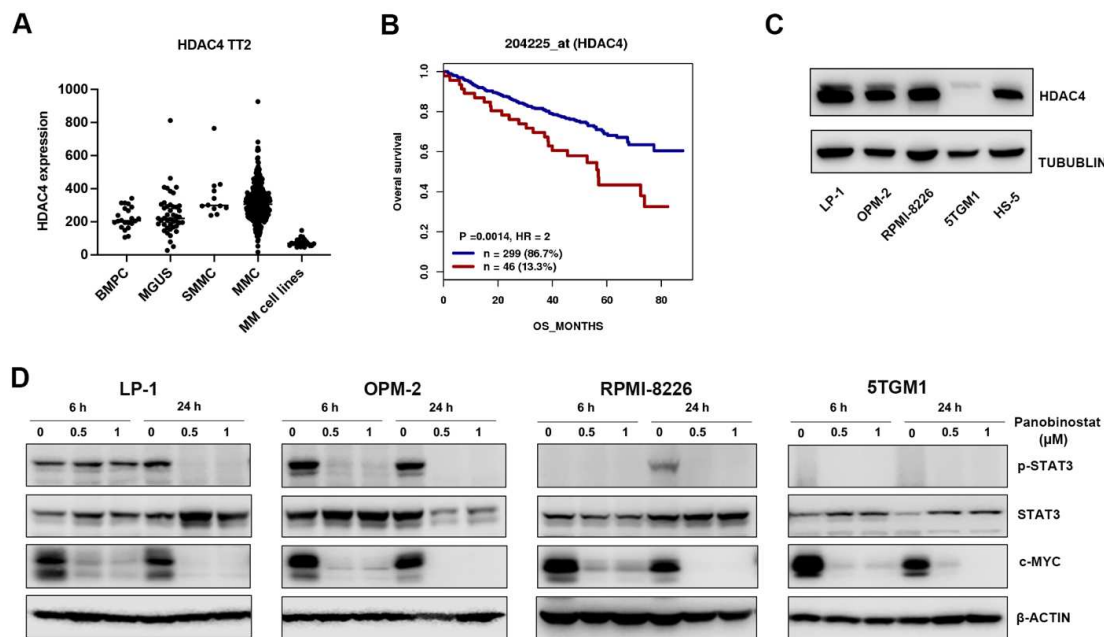
Supplemental Figure 1. Tasquinimod has no effect on MM cell apoptosis. (A) MM cell lines (LP-1, OPM-2, RPMI8226, 5TGM1) were treated with tasquinimod (TasQ) (10, 25 μM) for 24 h and 48 h. Apoptosis was detected by flow cytometry using an Annexin V/7-AAD staining and representative figures (including gating strategy) are shown. (B) MM cell lines were treated with tasquinimod (10, 25 μM) for 3 and 5 days. Apoptosis was analysed by Annexin V/7-AAD staining (n=4). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, Error bars indicate SD, Ordinary one-way ANOVA.



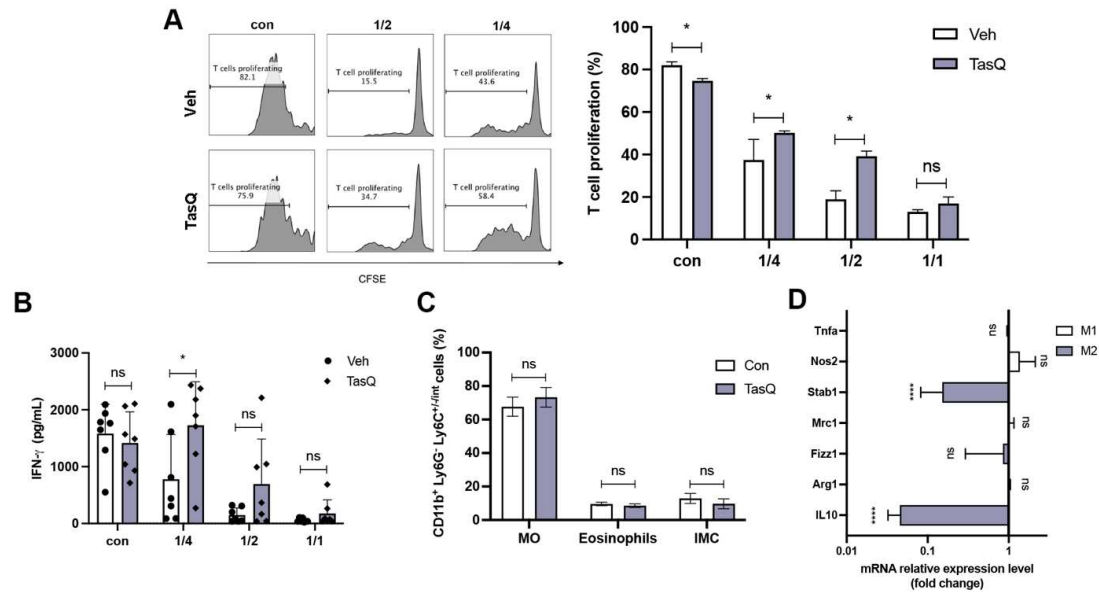
Supplemental Figure 2. Tasquinimod inhibits MM cell proliferation. MM cell lines (LP-1, OPM-2, RPMI-8226 and 5TGM1) and the stromal cell line HS-5 were treated with tasquinimod (TasQ) (10, 25 μ M) for 24 h and 48 h. Cell proliferation was analyzed using BrdU staining and flow cytometry. Representative figures and the gating strategy are shown for MM cell lines (A) and HS-5 cells (B).



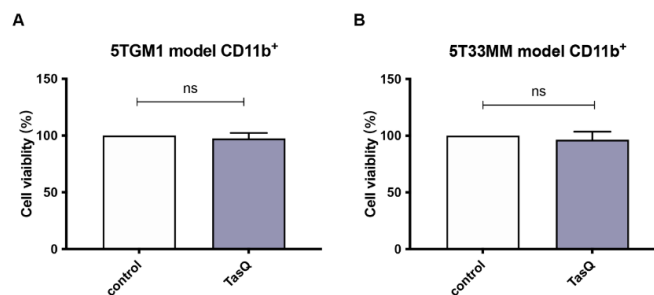
Supplemental Figure 3. Gene profile analysis in LP-1 cells after tasquinimod treatment. Volcano plot (left) comparing vehicle versus tasquinimod treatment. Heat map (right) showing differential gene expression in top-ranked genes in LP-1 cells after treatment with either 25 μ M of tasquinimod (Tas) or DMSO (cnt) for 6 h (A) and 24 h (B). The experiments were done in 3 biological tripates and represented by 3 columns for each condition.



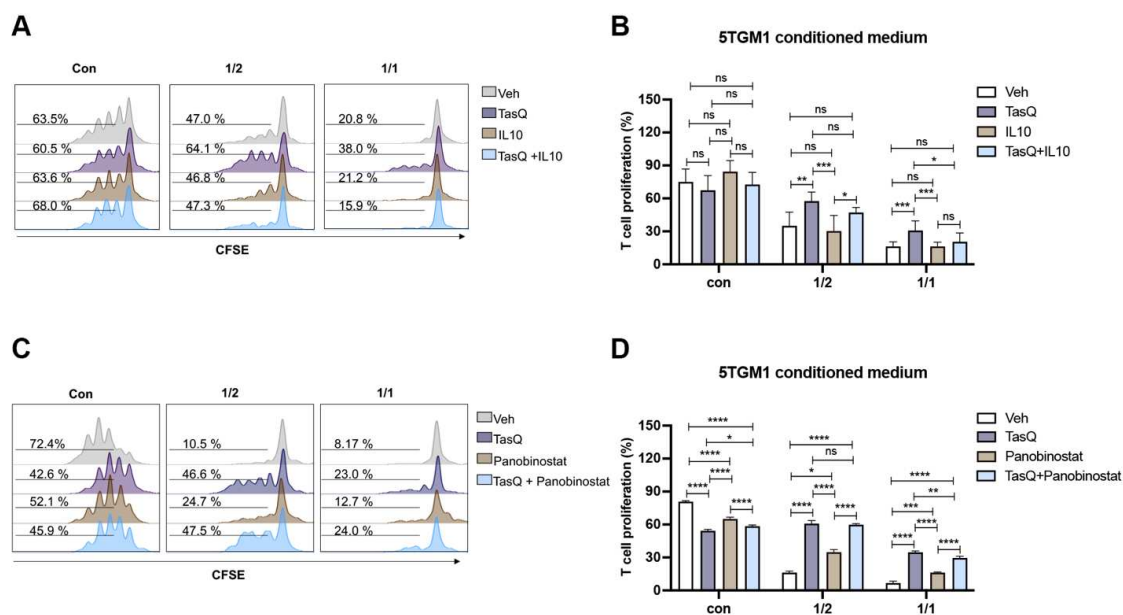
Supplemental Figure 5. HDAC4 correlates with a poor prognosis and targeting of histone deacetylases using panobinostat results in a similar decrease of c-MYC and p-STAT3. (A) The scatter plot of HDAC4 expression in BMPC (n=22), MGUS (n=44), SMMC (n=12), MMC (n=345), primary MM cell lines (n=23) samples using gene expression profiling (GEP) data using the online web tool Genomicscape. (B) Kaplan-Meier curve show the correlation of HDAC4 expression with overall survival (OS) in the TT2 MM patient cohort. High and low scores were defined as above and below the median level of expression, respectively. Log-rank test p values are indicated on the curves ($p = 0.0014$). (C) The HDAC4 expression in different MM cell lines and the stromal cell line HS-5 was detected by western blot (n=3). (D) LP-1, OPM-2, RPMI-8226 and 5TGM1 cells were cultured with panobinostat (0.5, 1 μM) for 6 h and 24 h. Whole-cell lysates were subjected to western blot using anti-phospho-STAT3, anti-STAT3, anti-c-MYC and anti- β -ACTIN antibodies (n=3).



Supplemental Figure 6. Tasquinimod reduces the MDSC suppressive capacity in 5T33MM-derived conditioned medium *in vitro*. (A) MACS sorted CD11b⁺ bone marrow cells were co-cultured in the presence of 5T33MM conditioned medium, CD3/CD28 microbeads and splenic CFSE-labeled T cells of naive mice. MDSC and T cells were co-cultured at a ratio of 1/4, 1/2 and 1/1 respectively. After 72 h, T cell proliferation was analyzed using flow cytometry (n = 3, Mann-Whitey U test). (B) Supernatant was collected from this assay and IFN- γ secretion was analyzed using ELISA (n=7, Mann-Whitey U test). (C) CD11b⁺ cells were sorted from the bone marrow of the 5T33MM model and treated with vehicle or tasquinimod (TasQ) for 24 h. The MDSC phenotype was evaluated via flow cytometry (n=3, Mann-Whitey U test). (D) CD11b⁺ cells were sorted from the bone marrow of the 5T33MM model and treated with vehicle or tasquinimod for 6 h. The mRNA level of genes was measured with RT-qPCR and calculated with the $\Delta\Delta C$. The data are expressed relative to their respective controls set to 1 (n=4, Unpaired t-test). * $p < 0.05$; **** $p < 0.0001$, Error bars indicate SD.



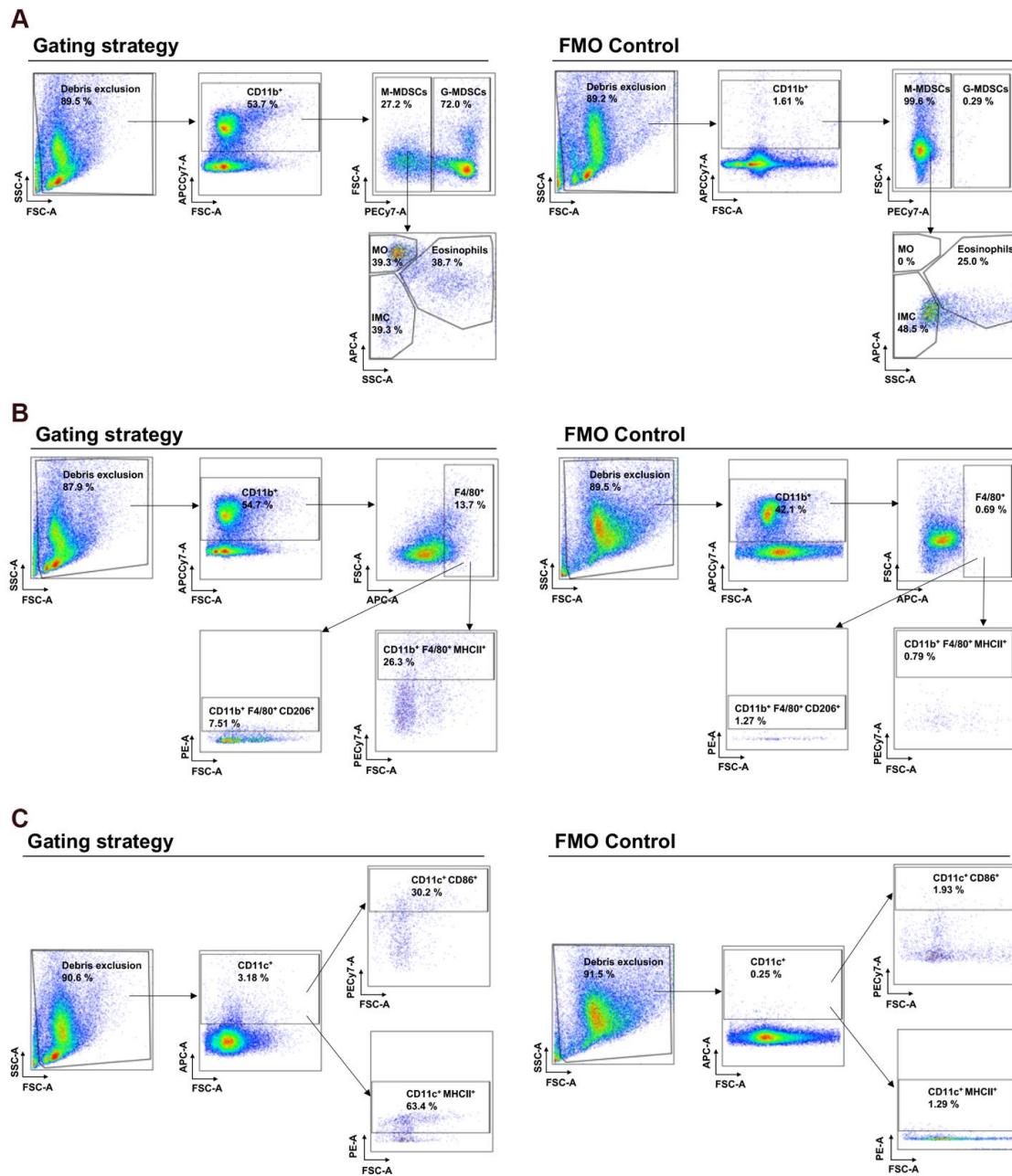
Supplemental Figure 7. Tasquinimod has no direct effect on the cell viability of MM-derived MDSC *in vitro*. MACS-sorted CD11b⁺ cells from the 5TGM1 (A) and 5T33MM (B) model were treated with/without tasquinimod (TasQ) (25 μM) for 24 h and cell viability was analyzed (n=4). Unpaired t-test. Error bars indicate



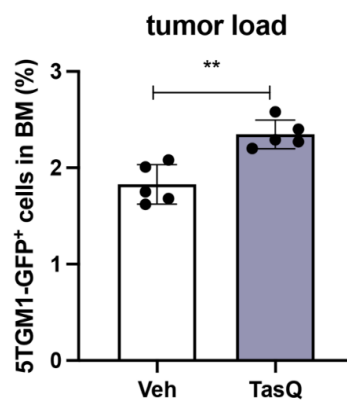
SD.

Supplemental Figure 8. Recombinant IL-10 and panobinostat change the T cell proliferation capacity in the presence of MDSC, with/without the treatment of tasquinimod. MACS sorted CD11b⁺ bone marrow cells were co-cultured in the presence of 5TGM1 conditioned medium, CD3/CD28 microbeads and splenic CFSE-labeled T cells of naïve mice, with/without tasquinimod (TasQ) (25 μM). MDSC and T cells were co-cultured at a ratio of 1/2 and 1/1 respectively. (A-B) Recombinant IL-10 was added at a concentration of 100 ng/mL and T cell proliferation was analyzed after 72 h (n=6). (C-D) Panobinostat was added to the assay at a concentration

of 10 nM and T cell proliferation was analyzed after 72 h (n=3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, One-way ANOVA. Error bars indicate SD.



Supplemental Figure 9. The flow cytometry gating strategy and FMO controls of myeloid cell populations in the 5TGM1 model. (A) CD11b⁺ cells, M-MDSCs, G-MDSCs, Ly6C^{hi} inflammatory monocytes (MO), Ly6C^{intermediate} eosinophils, and Ly6C^{low} immature myeloid cells (IMC). (B) Macrophages. (C) Dendritic cells.



Supplemental Figure 10. The percentage eGFP positive tumor cells in the bone marrow of tasquinimod-treated 5TGM1 mice after 10 days. 6-week-old C57BL/KaLwRij mice were inoculated with 1.0×10^6 5TGM1-eGFP cells on day 0 and treatment with tasquinimod (TasQ, 30 mg/kg in daily drinking water) started on day 1 (n=5/group). At day 10, all mice were sacrificed to investigate immune cell populations using flow cytometry. ** $p < 0.01$, Error bars indicate SD, Mann-Whitey U test.