Appendix

Primer Sequences

Differentially expressed transcripts, Wild type versus RARα$^{-/-}$ F9 cells (ligand independent)

Differentially expressed genes, ATRA versus vehicle treated Wild type and RARα$^{-/-}$ F9 cells

Exogeneous RARα Expression in F9 Wt cells

Table S1a. Construction and Sequencing Primers

Forward	Sense primer (5′-3′)	**Reverse**	Antisense primer (5′-3′)	**Product**
mPML(+)MN	tttcaattgCCatgGAAACTGAACCAGTTTCCGT	mPML(-)TH	tttaagcttGACTGGGTCGTTTCCCCTGTGTCACT	1696bp
mRARα2A(+)M	TTTcaattgCCatgTACGAGAGTGTGGAAGTCG	mRARα2B(-)TX	TTTCTCGAga**CtggGTCTCGATGGagtgg**	183bp
mRARαC(+)MT	tttcaattgCCatggagACcCAGtcCAGCAGTTCC	mRARαE(-)XH	tttaagcttactcgagCATTTCCTGGATCA	1058bp
mRARαF(+)X	tttctcgagAACTCTGAGGGCTT	mRARαF(-)H	tttaagctTCATGGGGATTGGGTGGCT	147bp
mRARα(+)ΔT	ACCGACTTGGTgTTTGCCTTCGC	mRARα(-)ΔT	GCGAAGGCAAAcACCAAGTCGGT	na
MCS(+)	AATTCCAATTGAACTCGAGTTAAGCTTA	MCS(-)	GATCTAAGCTTAACTCGAGTTCAATTGG	na
T7(+)	TAATACGACTCACTATAGGGCGA	SP6(+)	ATTTAGGTGACACTATAGAATAC	na

M; MfeI, N; NcoI, T; Tth111I, H; HindIII, X; XhoI. MCS; multiple cloning site. (+); sense primer, (-); antisense primer. Letters not capped indicate non-complementary bases with specific functions in the resulting PCR product (na: not applicable).

Table S1b. Gene Expression Primers

Forward	Sense primer (5′-3′)	**Reverse**	Antisense primer (5′-3′)	**Product**	(bp)
Induced	by RA			cDNA	gDNA
mHoxb2(+)A	AGCCCCCGAGTTCCCCTGGATGA	mHoxb2(-)B	CTCCAGCTCCAGCAGTTGCGTGTTG	207	8385
mCyp26a1(+)A	GAAACATTGCAGATGGTGCTTCAG	mCyp26a1(-)B	CGGCTGAAGGCCTGCATAATCAC	272	728
mHoxb5(+)A	GCGAGCCAGCTAAGCAGCCCCA	mHoxb5(-)D	AGGCGGTCCGGGCCCTTTTTCCG	166	870
mHoxa5(+)C	CCCCTGGATGCGCAAGCTGCACATT	mHoxa5(-)F	TTCTCCAGCTCCAGGGTCTGGTAGCGA	105	1062
Downregulated	in RARα$^{-/-}$				
mMest(+)E	CAATCCTGCGGCGGGCGGCATGGGA	mMest(-)F	GGTAGAAGATGCGTAGGCCTTTGTAGGT	213	2547
mTex13(+)A	GTGTTCCTGTCACTTCTGTCGCCCT	mTex13(-)B	GCACAGGCTTCCTGAACATCCCGA	281	530
mHmgcs1(+)A	GCCCTGGACCGCTGCTATTCT	mHmgcs1(-)B	AGTCATTCAGGAACATCCGAGCTAGA	166	1460
mGab1(+)A	CCAACTCGCCACCTCGACAACA	mGab1(-)B	GAGAGTCGCTGCTCGATGTCGTA	393	10252
mTcfap2a(+)A	TTACCTCACGCCATCGAGGACGT	mTcfap2a(-)B	GCTGTTGGACTTGGACAGGGACA	108	5075
mBcl11a(+)C	CCTCTCCTCGGTCTGCACACGGA	mBcl11a(-)D	GAAACCATGCACTGGTGAATGGCTGT	141	4932
Upregulated	in RARα$^{-/-}$				
mSlc38a4(+)A	CCTTCTTGCGGCCCTCTTTGGTTAT	mSlc38a4(-)B	CAGCACGATGGGCACGGTCAGT	157	2625
mStmn2(+)C	TCCTGCTTCTACCCGGAGCCGCG	mStmn2(-)D	CCAGCTTTTTCTGAATCTCCTCCAGA	153	2435
mRpL39l(+)A	GCGGTGCAGGGCTCACA	mRpL39l(-)B	TCCATTGTGGAATGGGACGAT	203	4082
mRef2L(+)C	TACAAAGAGAGCAGTCTGCCGTGA	mRef2L(-)B	ATGGGTTCCGTTCTTGTGCAGCT	913	1781
mMobp(+)A	GGCCCGCTATCCACAGGAACCT	mMobp(-)B	TCTGGCAGGCACAGCAGATCCAGT	327	18251
mRlf(+)A	CAGCAGCTCCAAACGGCCAGTGTA	mRlf(-)B	CATTTCCTCATCTTCACTTGATCTGAGTT	258	8285
Controls					
m36B4(+)A	AGAACAACCCAGCTCTGGAGAAA	m36B4(-)B	ACACCCTCCAGAAAGCGAGAGT	448	629
mHPRT1(+)A	GCTTGCTGGTGAAAAGGACCTCTCGAAG	mHPRT1(-)B	CCCTGAAGTACTCATTATAGTCAAGGGCAT	117	288
Genotyping					
mRARαE6(+)	TCAGAGAGCTACACGCTGACG	mRARαE7(-)	ATGGTGAGGGTGGTGAAGCCG	239	414
mRARaE34(+)	TGGCTCAAACCACTCCATCGAGA	mRARαE6(-)	CCTGGTGCGCTTTGCGAACC	425	3944
mRARβ2A(+)	tttcaattgCCatgTTTGACTGTATGGATGTTCT	mRARβE4(-)	CACTGACGCCATAGTGGTA	289	26584
mRARγ2A(+)	tttcaattgCCatgTACGACTGCATGGAATCGT	mRARγE7(-)	TTGCTGACCTTGGTGATGAGTT	551	6031
rβ-globin5'C	TATTCCAGAAGTAGTGAGGAGGC	rβ-globin3'B	CGCCCTATAGTGAGTCGTATTACA	162	735

Table S1c. Bisulfite Sequencing primers

Forward	Sense primer (5′-3′)	**Reverse**	Antisense primer (5′-3′)	**Product**	**Position**
mMestCpG(+)G	GTTTTTTAGGGTTTAGGGATTAGTG	mMestCpG(-)H	CAACAAAAACAACAAACAACAACTC	453	-337;+116
mMestCpG(+)G	GTTTTTTAGGGTTTAGGGATTAGTG	mMestCpG(-)J	CTTTAAATAAAAATTTTTACCTCCC	695	-337;+358
mStmn2CpG(+)I	GAGAGTTAATTTGTTTGAGTTTTTG	mStmn2CpG(-)J	ATTCCTATCATAAATATCTTTCCCTTTATA	324	-103;+224
mStmn2CpG(+)I	GAGAGTTAATTTGTTTGAGTTTTTG	mStmn2CpG(-)L	TTCCCCCTTTAATCTCTTAAAACTC	368	-103;+268
mTex13CpG(+)E	TTTTGTTTTTAATGGGTTTTAATAAATAGT	mTex13CpG(-)F	ACAAAAATAACAAAAACACAAAACC	277	-248;+29
mSlc38a4CpG(+)I	TTTGGAAAGATGTTTTTGTTGATAG	mSlc38a4CpG(-)F	CCTACCCTAATTTACTTCACTTCTAATCA	497	-123;+374
mCyp26a1CpG(+)E	TGAATTAATTTGTTTGATTAAGGTAA	mCyp26a1CpG(-)F	ACAATACAAATCCCAAAACTTAAAC	219	-67;+152

Table S1d. ChIP primers

Forward	Sense primer (5′-3′)	**Reverse**	Antisense primer (5′-3′)	**Product**	**Position**
mMest(+)G	GTCTTCCAGGGTCTAGGGACCAGTG	mMest(-)N	TCTCTAATCCTGAACCCCAGATTCTAGT	140	-337;-197
mSlc38a4(+)I	CCTGGAAAGATGTTTCTGTTGACAG	mSlc38a4(-)J	GTTAGGATCTAGCCCTAAAATTCCAC	277	-123;+171
mStmn2(+)I	GAGAGCCAATCTGCTTGAGCTTCTG	mStmn2(-)L	GGAGAGAGCGAGAGAGGAGTCTGAGT	125	-103;+22
mTex13(+)I	TGCCAGTCCTGGAAGTTGGAACAGT	mTex13(-)H	GCGACAGAAGTGACAGGAACACAAGACC	164	-132;+32
mCyp26a1-R1(+)A	CCCGATCCGCAATTAAAGATGA	mCyp26a1-R1(-)B	CTTTATAAGGCCGCCCAGGTTAC	87	-97;-10

Table S2a. Increased Expression in F9 RARα$^{-/-}$ cells (2.7 fold or more relative to Wt)

8h atRA		EtOH						
Fold Change	P-value	Fold Change	P-value	Gene Symbol	Gene ID	Gene Title	Location	Tissue / Process
11.09	0.00087	10.35	0.00142	**Slc38a4**	AK003626	solute carrier family 38, member 4	chr15	Aminoacid transporter
9.991	0.00083	10.25	0.00090	**Slc38a4**	AK003626	solute carrier family 38, member 4	chr15	and Fetal Liver Dev.
5.915	0.00012	6.622	0.00044	RpL39l	AK005645	ribosomal protein L39-like	chr16	Testis expressed
7.073	n.s,	5.726	n.s,	Ref2L/SREFL	BG071029	similar to RNA and export factor binding protein 1-II	chr10	
5.339	0.00884	5.533	0.01190	**Stmn2**	BM115022	stathmin-like 2	chr3	Neurogenesis, DRG
4.395	0.00329	4.665	0.00726	**Stmn2**	BM115022	stathmin-like 2	chr3	and Fetal Brain Dev.
3.236	0.01630	4.108	0.01470	Mobp	BM899593	myelin-associated oligodendrocytic basic protein	chr9	Neurogenesis
2.860	0.00238	3.192	0.00267	Mobp	BM899593	myelin-associated oligodendrocytic basic protein	chr9	and Spinal cord
2.926	0.00143	3.412	*0.01740*	Hook1	AV240656	hook homolog 1 (Drosophila)	chr4	Testis expressed
3.006	n.s,	3.036	*0.03330*	Chd2	BE199465	chromodomain helicase DNA binding protein 2	chr7	Brain, Pituitary
4.431	n.s,	3.007	*0.02070*	Rlf	BB704706	rearranged L-myc fusion sequence	chr4	Placental factor
2.246	n.s,	2.990	*0.04250*	Atrx	BB825830	alpha thalassemia/mental retardation syndrome X-linked homolog	chrX	Mental retardation
2.737	0.00791	2.941	0.00795	Gcm1	NM_008103	glial cells missing homolog 1 (Drosophila)	chr9	Placental factor
2.776	n.s,	2.892	0.00619	2700049A03Rik	BM230224	RIKEN cDNA 2700049A03 gene	chr12	Testis expressed
3.029	0.01100	2.838	0.00737	Nhedc1	AK015318	Na+/H+ exchanger domain containing 1	chr3	Testis expressed
2.694	0.01010	2.584	0.00352	Nhedc1	AK015318	Na+/H+ exchanger domain containing 1	chr3	Testis expressed
2.834	0.00075	2.821	0.00132	Sema3e	Z93948	sema domain, immunoglobulin domain (Ig)	chr5	Placental factor
2.687	0.00736	2.369	0.00130	Sema3e	Z93948	sema domain, immunoglobulin domain (Ig)	chr5	Placental factor

Table S2b. Decreased Expression in F9 RARα$^{-/-}$ cells (0.5 fold or less relative to Wt)

8h atRA		EtOH						
Fold Change	P-value	Fold Change	P-value	Gene Symbol	Gene ID	Gene Title	Location	Tissue / Process
0.122	0.01020	0.113	0.00731	**Mest**	AW555393	mesoderm specific transcript	chr6	Placental factor
0.128	0.00006	0.143	0.00000	Gab1	NM_021356	growth factor receptor bound protein 2-associated protein 1	chr8	Placental factor, DRG
0.143	0.00004	0.144	0.00001	Gab1	NM_021356	growth factor receptor bound protein 2-associated protein 1	chr8	and Spinal cord
0.144	0.00014	0.148	0.00001	Gab1	NM_021356	growth factor receptor bound protein 2-associated protein 1	chr8	
0.281	0.00564	0.294	0.00002	**Tex13**	AK016637	testis expressed gene 13	chrX	Testis expressed
0.303	0.00002	0.325	0.00009	**Tex13**	AK016637	testis expressed gene 13	chrX	And Placental factor
0.498	0.00345	0.375	0.01130	Bcl11a	BF731393	B-cell CLL/lymphoma 11A (zinc finger protein)	chr11	B-cell, fetal brain
0.411	0.01880	0.389	0.01050	Tcfap2a	NM_011547	transcription factor AP-2, alpha	chr13	Neurogenesis, placenta
0.339	0.00225	0.395	0.00263	LemD1vF	AI842757	RIKEN cDNA 6430514M23 gene	chr1	Testis expressed
0.458	0.00966	0.404	0.00408	LemD1vF	AI849139	RIKEN cDNA 6430514M23 gene	chr1	
0.408	0.00004	0.445	0.00088	Cux1	BC014289	cut-like homeobox 1	chr5	Placenta/Testis
0.440	0.03010	0.446	0.00019	Hmgcs1	BB705380	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	chr13	Reductase function
0.453	0.01440	0.472	0.00036	Hmgcs1	BB705380	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	chr13	Bronchial epithelium
0.455	0.00196	0.495	*0.01950*	Hmgcs1	BB705380	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	chr13	And Fetal liver.
0.405	0.00450	0.458	0.00170	Ildr1	BG084606	immunoglobulin-like domain containing receptor 1	chr16	
0.491	0.02640	0.460	0.00118	Dars2	BM228846	aspartyl-tRNA synthetase 2 (mitochondrial)	chr1	
0.446	0.00050	0.461	0.00051	Mmp11	NM_008606	matrix metallopeptidase 11	chr10	Placental factor
0.459	0.00478	0.484	0.00266	Spire1	AU067702	spire homolog 1 (Drosophila)	chr18	Brain
0.343	0.00056	0.491	*0.01810*	Hoxb5	NM_008268	homeo box B5	chr11	
0.436	0.00350	0.528	n.s,	Ebf1	BB125261	early B-cell factor 1	chr11	B-cell
0.473	0.02540	0.532	n.s,	Ebf1	BB125261	early B-cell factor 1	chr11	B-cell
0.498	0.01380	0.558	n.s,	Ebf1	BB125261	early B-cell factor 1	chr11	B-cell

Differentially Expressed Transcripts identified by Microarray Analysis of F9 Wt and RARα$^{-/-}$ cells. Fold Change denotes the expression in F9 RARα$^{-/-}$ relative to Wt cells upon 8 hr exposure of either RA or vehicle. Gene symbols and titles are in accordance with NCBI nomenclature. Gene symbols in bold indicate that the transcript levels have been validated by real-time PCR. Multiple entrez of a gene indicate differential detection with multiple probes. Expression of the respective genes was mapped to specific tissues based on references presented in the paper and on expression data deposited in BioGPS, The Gene Portal Hub (http://biogps.gnf.org). DRG: dorsal root ganglia.

Table S3. Fold increase in transcript level in F9 Wt and RARα$^{-/-}$ cells upon RA treatment

3.0 fold or more upon 8h RA treatment of Wt or RARα$^{-/-}$ cells (relative to vehicle treated cells)

RARα$^{-/-}$:Wt		Wt		RARα$^{-/-}$			Public ID	Gene Title	Location
Ratio of Fold Change	Average	Fold Change	P-value	Fold Change	P-value	Gene Symbol			
0.71	4.0	4.690	0.00115	3.319	0.05387	**Hoxb5**	NM_008268	homeo box B5	chr11
0.79	4.0	4.468	0.00312	3.534	0.00001	Cdx1	BC019986	caudal type homeo box 1	chr18
0.69	3.6	4.226	0.00054	2.936	0.00069	Csn3	NM_007786	casein kappa	chr5
0.74	3.6	4.162	0.00011	3.094	0.00164	Tmtc1	AV341449	transmembrane and tetratricopeptide containing 1	chr6
0.70	3.4	4.023	0.00049	2.819	0.00035	Aurkc	NM_020572	aurora kinase C	chr7
0.75	3.3	3.780	0.01960	2.825	0.00027	MP11	BC005730	RIKEN cDNA A330049M08 gene	chr4
0.70	2.9	3.392	0.00165	2.385	0.00002	Rarb	BB266455	retinoic acid receptor. beta	chr14
0.78	2.9	3.280	0.01380	2.559	0.00065	Pdgfrb	AA499047	platelet derived growth factor receptor, b polypeptide	chr18
0.66	2.8	3.326	0.00432	2.184	0.00340	**Hoxb2**	NM_134032	homeo box B2	chr19
1.03	3.2	3.141	0.00785	3.226	0.00002	**Cyp26a1**	NM_007811	cytochrome P450. family 26, subfamily a, polypeptide	chr19
1.07	2.9	2.844	0.00760	3.045	0.02380	**Hoxa5**	BC011063	homeo box A5	chr6

RA induced Transcripts identified by Microarray Analysis of F9 Wt and RARα$^{-/-}$ cells. Fold Change denotes the transcript level in RA treated cells relative to vehicle treated cells. Gene symbols and titles are in accordance with NCBI nomenclature. Gene symbols in bold indicate that the transcript levels have been validated by real-time PCR to be induced by RA to a similar extent in F9 Wt and RARα$^{-/-}$ cells. Ratio of Fold Change indicates the fold induction in RARα$^{-/-}$ cells relative to the fold induction in Wt cells. Average indicates the average fold induction between RARα$^{-/-}$ and Wt cells

Construction of pSG5 RARα and pSG5 PML-RARα

A multiple cloning site was introduced into EcoRI opened and alkaline phosphate treated pSG5, mammalian expression vector (Cat. #216201, Stratagene, CA) by insertion of the annealed MCS(+) and MCS(-) oligos through a T4 ligation reaction (Cat. #M0202, New England Biolabs, MA). This introduced unique HindIII and XhoI cloning sites, thus generating pSG5 MCS. Fragments mPMLΔCT, mRARα2AB, mRARαCE, and mRARαF were generated by Phusion high fidelity PCR reactions (Cat. #F-530, New England Biolabs, MA) on cDNA from F9 Wt cells using mPML(+)MN/mPML(-)TH, mRARαC(+)MT/mRARαE(-)XH and mRARαF(+)X/mRARαF(-)H primer pairs, respectively. The RARαBE fragment and pSG5 MCS were digested with MfeI/HindIII and EcoRI/HindIII, respectively, gel purified, and ligated to generate pSG5 mRARαCE. An undesired Tth111I restriction site was removed using the mRARα(+)ΔT/mRARα(-)ΔT primer pair in a Quickchange protocol (Cat. #200518, Stratagene, CA), thus generating pSG5 mRARαCDΔT devoid of Tth111I sites. Notably, the introduced mutation is silent and consequently, no change in amino-acid sequence results from the introduced mutation. The RARαF fragment and pSG5 mRARαCEΔT were both digested with XhoI/HindIII, gel purified, and ligated to generate pSG5 mRARαCF. The RARα2AB fragment and pSG5 mRARαCF were both digested with MfeI/Tth111I, gel purified, and ligated to generate pSG5 mRARα2AF for expression of full-length RARα2. The PMLΔCT fragment and pSG5 MCS were digested with MfeI/HindIII and EcoRI/HindIII, respectively, gel purified, and ligated to generate pSG5 mPMLΔCT. The pSG5 mPMLΔCT and pSG5 mRARαCF were both digested with Tth111I/XbaI, gel purified and ligated to generate pSG5 mPML-RARα (see the Appendix for complete aminoacid sequence of the expressed transgene). The sequences of the primers are listed in the appendix. The ligated products were each transformed into competent DH5α bacteria and plated on ampicillin containing LB agar plates for overnight incubation at 37°C. DNA was isolated from resistant colonies and confirmed by restriction analysis to contain inserts of the expected sizes. In addition, the coding region of each construct was verified by sequencing to contain the desired DNA sequence.

Amino-acid Sequence of the PML-RARα fusion protein

PML region (residues 1-500 of PML)

```
METEPVSVQKVPAPPGSPCRQQDSALTPTPTMPPPEEPSEDYEHSQSPAEQAIQEEFQFLRCPSCQAQAKCPKLLPCLHTLCSGCLEAPGLQCPICK
APGQADANGEALDNVFFESLQRRLAVFRQIVDAQAACTRCKGLADFWCFECEQLICSKCFEAHQWYLKHEARPLADLRDNSVSSFLDSTRKSNIFCS
NTNHRNPALTDIYCRGCAKPLCCTCALLDRNHSHLHCDIGEEIQQWHEELGTMTQTLEEQGRTFDSAHAQMCSAIGQLDHARADIEKQIRARVRQVV
DYVQAQERELLEAVNDRYQRDYQEIAGQLSCLEAVLQRIRTSGALVKRMKLYASDQEVLDMHSFLRKALCSLRQEEPQNQKVQLLTRGFEEFKLCLQ
DFISCITQRINAAVASPEAASNQPEAASTHPVTTSTPEDLEQPKEVQSVQAQALELSKTQPVAMVKTVPGAHPVPVYAFSMQGPTYREEASQTVGSM
KRKCSHEDCSRKIIKMESTEENEDRLATSSPEQSWPSTFKATSPPHLDGTSNPESTVPEKKILLPNNNHVTSDTGET-
```

RARα region (residues 63-462 of RARα1)

```
TQSSSSEEIVPSPPSPPPLPRIYKPCFVCQDKSSGYHYGVSACEGCKGFFRRSIQKNMVYTCHRDKNCIINKVTRNRCQYCRLQKCFDVGMSKESVR
NDRNKKKKEAPKPECSESYTLTPEVGELIEKVRKAHQETFPALCQLGKYTTNNSSEQRVSLDIDLWDKFSELSTKCIIKTVEFAKQLPGFTTLTIAD
QITLLKAACLDILILRICTRYTPEQDTMTFSDGLTLNRTQMHNAGFGPLTDLVFAFANQLLPLEMDDAETGLLSAICLICGDRQDLEQPDKVDMLQE
PLLEALKVYVRKRRPSRPHMFPKMLMKITDLRSISAKGAERVITLKMEIPGSMPPLIQEMLENSEGLDTLSGQSGGGTRDGGGLAPPPGSCSPSLSP
SSHRSSPATQSP*
```

Alignment of PML-RARα with human PML and human RARα1

```
                    1                                                                                                  100
PML-RARaBF   (1)   METEPVSVQKVPAPPGSPCRQQDSALTPTPTMPPPEEPSEDYEHSQSPAEQAIQEEFQFLRCPSCQAQAKCPKLLPCLHTLCSGCLEAPGLQCPICKAPG
      hPml   (1)   METEPVSVQKVPAPPGSPCRQQDSALTPTPTMPPPEEPSEDYEHSQSPAEQAIQEEFQFLRCPSCQAQAKCPKLLPCLHTLCSGCLEAPGLQCPICKAPG
    hRARa1   (1)   ----------------------------------------------------------------------------------------------------

                    101                                                                                                200
PML-RARaBF (101)   QADANGEALDNVFFESLQRRLAVFRQIVDAQAACTRCKGLADFWCFECEQLICSKCFEAHQWYLKHEARPLADLRDNSVSSFLDSTRKSNIFCSNTNHRN
      hPml (101)   QADANGEALDNVFFESLQRRLAVFRQIVDAQAACTRCKGLADFWCFECEQLICSKCFEAHQWYLKHEARPLADLRDNSVSSFLDSTRKSNIFCSNTNHRN
    hRARa1   (1)   ----------------------------------------------------------------------------------------------------

                    201                                                                                                300
PML-RARaBF (201)   PALTDIYCRGCAKPLCCTCALLDRNHSHLHCDIGEEIQQWHEELGTMTQTLEEQGRTFDSAHAQMCSAIGQLDHARADIEKQIRARVRQVVDYVQAQERE
      hPml (201)   PALTDIYCRGCAKPLCCTCALLDRNHSHLHCDIGEEIQQWHEELGTMTQTLEEQGRTFDSAHAQMCSAIGQLDHARADIEKQIRARVRQVVDYVQAQERE
    hRARa1   (1)   ----------------------------------------------------------------------------------------------------

                    301                                                                                                400
PML-RARaBF (301)   LLEAVNDRYQRDYQEIAGQLSCLEAVLQRIRTSGALVKRMKLYASDQEVLDMHSFLRKALCSLRQEEPQNQKVQLLTRGFEEFKLCLQDFISCITQRINA
      hPml (301)   LLEAVNDRYQRDYQEIAGQLSCLEAVLQRIRTSGALVKRMKLYASDQEVLDMHSFLRKALCSLRQEEPQNQKVQLLTRGFEEFKLCLQDFISCITQRINA
    hRARa1   (1)   ----------------------------------------------------------------------------------------------------

                    401                                                                                                500
PML-RARaBF (401)   AVASPEAASNQPEAASTHPVTTSTPEDLEQPKEVQSVQAQALELSKTQPVAMVKTVPGAHPVPVYAFSMQGPTYREEASQTVGSMKRKCSHEDCSRKIIK
      hPml (401)   AVASPEAASNQPEAASTHPVTTSTPEDLEQ----------------------------------------------EASQTVGSMKRKCSHEDCSRKIIK
    hRARa1   (1)   ----------------------------------------------------------------------------------------------------

                    501                                                                                                600
PML-RARaBF (501)   MESTEENEDRLATSSPEQSWPSTFKATSPPHLDGTSNPESTVPEKKILLPNNNHVTSDTGETTQSSSSEEIVPSPPSPPPLPRIYKPCFVCQDKSSGYHY
      hPml (455)   MESTEENEDRLATSSPEQSWPSTFKATSPPHLDGTSNPESTVPEKKILLPNNNHVTSDTGETEERVVVISSSEDSDTENLSSHELDDSSSESSSLQLEGP
    hRARa1   (1)   MASNSSSCPTPGGGHLNGYPVPPYAFFFPPMLGGLSPPGALTTLQHQLPVSGYSTPSPATIETQSSSSEEIVPSPPSPPPLPRIYKPCFVCQDKSSGYHY

                    601                                                                                                650
PML-RARaBF (601)   GVSACEGCKGFFRRSIQKNMVYTCHRDKNCIINKVTRNRCQYCRLQKCFDVGMSKESVRNDRNKKKKEAPKPECSESYTLTPEVGELIEKVRKAHQETFP
      hPml (555)   NSLKALDESLAEPHLEDRTLVFFDLKIDNETQKISQLAAVNRESKFRVLIQPEAFSVYSKAVSLEAGLRHFLSFLTTMHRPILACSRLWGPGLPIFFQTL
    hRARa1 (101)   GVSACEGCKGFFRRSIQKNMVYTCHRDKNCIINKVTRNRCQYCRLQKCFEVGMSKESVRNDRNKKKKEVPKPECSESYTLTPEVGELIEKVRKAHQETFP

                    701                                                                                                800
PML-RARaBF (701)   ALCQLGKYTTNNSSEQRVSLDIDLWDKFSELSTKCIIKTVEFAKQLPGFTTLTIADQITLLKAACLDILILRICTRYTPEQDTMTFSDGLTLNRTQMHNA
      hPml (655)   SDINKLWEFQDTISGFLAVLPLIRERIPGASSFKLGNLAKTYLARNMSERSALASVLAMRDLCCLLEISPGLPLAQHIYSFSSLQCFASLQPLIQASVLP
    hRARa1 (201)   ALCQLGKYTTNNSSEQRVSLDIDLWDKFSELSTKCIIKTVEFAKQLPGFTTLTIADQITLLKAACLDILILRICTRYTPEQDTMTFSDGLTLNRTQMHNA

                    801                                                                                                900
PML-RARaBF (801)   GFGPLTDLVFAFANQLLPLEMDDAETGLLSAICLICGDRQDLEQPDKVDMLQEPLLEALKVYVRKRRPSRPHMFPKMLMKITDLRSISAKGAERVITLKM
      hPml (755)   QSEARLLALHNVSFVELLNAYRTNRQEGLKKYVHYLSLQTTPLSSSASTQVAQFLQALSTHMEGLLEGHAPAGAEGKAESKGCLA---------------
    hRARa1 (301)   GFGPLTDLVFAFANQLLPLEMDDAETGLLSAICLICGDRQDLEQPDRVDMLQEPLLEALKVYVRKRRPSRPHMFPKMLMKITDLRSISAKGAERVITLKM

                    901                                                         962
PML-RARaBF (901)   EIPGSMPPLIQEMLENSEGLDTLSGQSGGGTRDGGGLAPPPGSCSPSLSPSSHRSSPATQSP*
      hPml (840)   --------------------------------------------------------------
    hRARa1 (401)   EIPGSMPPLIQEMLENSEGLDTLSGQPGGGRDGGGLAPPPGSCSPSLSPSSNRSSPATHSP*
```

Alignment of the predicted aminoacid sequence of the generated PML-RARα fusion with polypeptide sequences of the human PML (Acc# AAI39796.1) and human RARα1 (Acc# NP_000955.1). Note that the gap in the alignment between residues 431 and 476 of the fusion protein is caused by murine specific skipping of PML exon 5.