

1 **SUPPLEMENTAL LEGENDS**

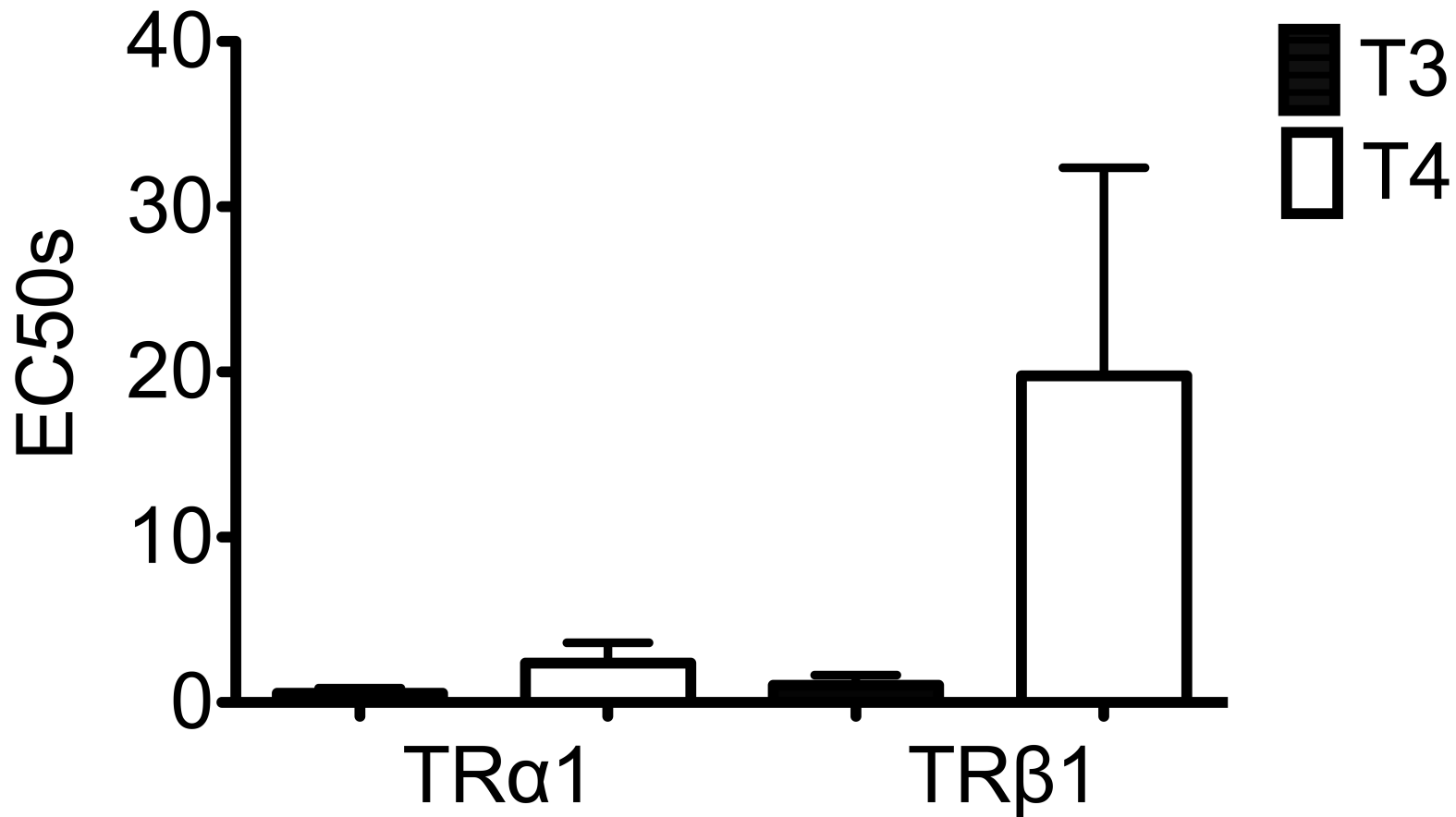
2 **Supplemental Table S1.** EC<sub>50</sub>'s with 95% confidence intervals. All experiments include S.E.M for at  
3 least 3 replicates. Also included in the table is the change in the EC<sub>50</sub>'s observed between T<sub>3</sub> versus T<sub>4</sub>.

4  
5 **Supplemental Figure S1.** Representative EC<sub>50</sub>s/affinities graphed with 95% confidence intervals for  
6 protease protection affinity of TR isoforms with T<sub>3</sub> and T<sub>4</sub> (as in Figure 1).

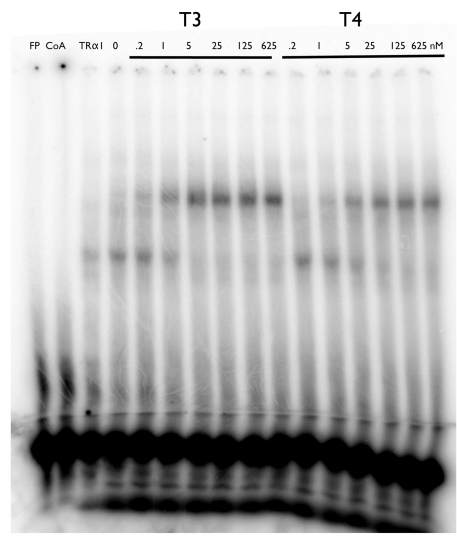
7  
8 **Supplemental Figure S2. Representative raw electrophoretic mobility supershift data for TRα1 and**  
9 **TRβ1 with (A) DR4:TRAP220, (B) DR4:SRC1, or (C)DIV6:NcoRω.** Assay was conducted as  
10 described as in Figure 8. FP = free probe; coA or coR = addition of coregulator indicated.

11  
12 **Supplemental Figure S3. T<sub>4</sub> regulates endogenous gene expression.** GH3 cells stably transformed with  
13 TRα1 (64) were placed in DMEM:F12 (1:1) and 5% Hormone-Stripped FBS for 24 hours containing the  
14 indicated concentrations of T<sub>3</sub> or T<sub>4</sub>. RNA was then isolated using Trizol-chloroform extraction and a  
15 cDNA library was prepared using Qiagen Quantitect-RT from 1μg of RNA per reaction. qrtPCR was then  
16 conducted using a Roche Lightcycler 480 SYBR green MasterMix and a Roche Lightcycler 480 with  
17 primers (5'-CTTCGAGTGACTGAGGGCTG and 5'-CCACGGAGTTCTCTGCTTTCA) and (5'-  
18 CACCGAATCTGGGTCGAGTC and 5'-CCGTTACCTGTATGCACTC ) to determine the expression  
19 levels of Sonic Hedge Hog (SHH) and Kruppel-Like Factor 9 (KLF9); expression of each was normalized  
20 against the housekeeping gene 60S ribosomal protein L8 (RLP8). The mean and standard errors (n=3) are  
21 shown.

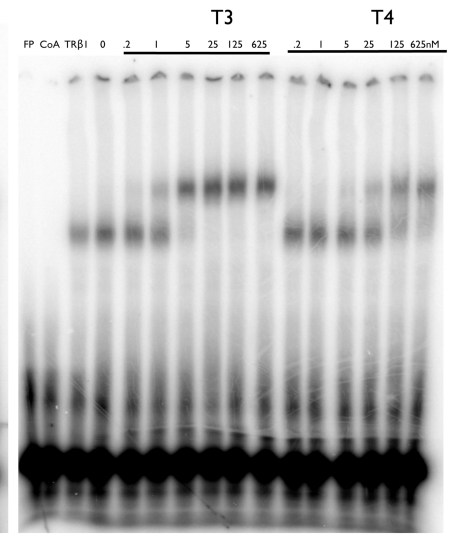
# Protease Protection



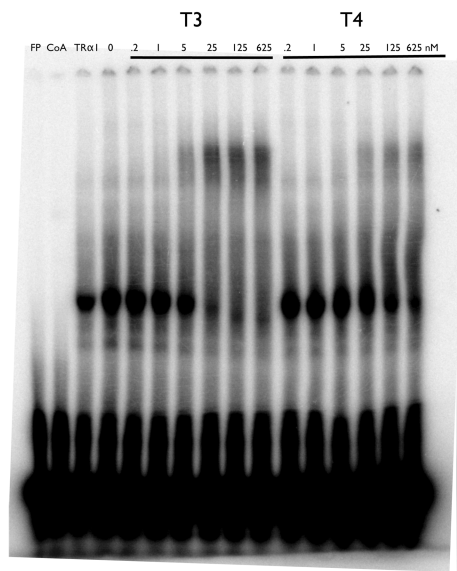
A.  
DR4:TRAP220:TR $\alpha$ I



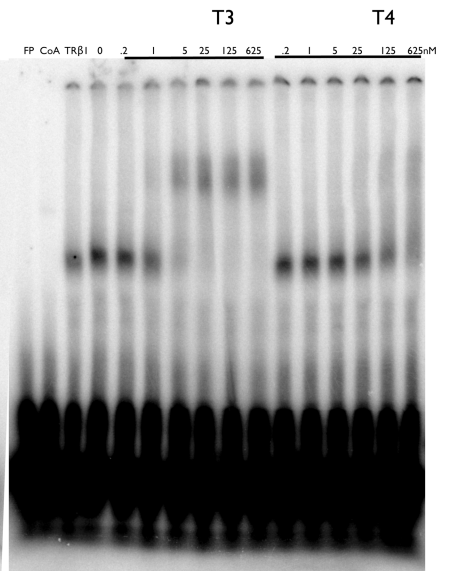
DR4:TRAP220:TR $\beta$ I



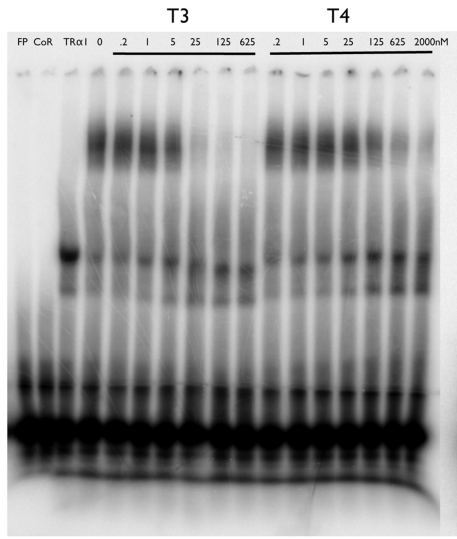
B.  
DR4:SRC1:TR $\alpha$ I



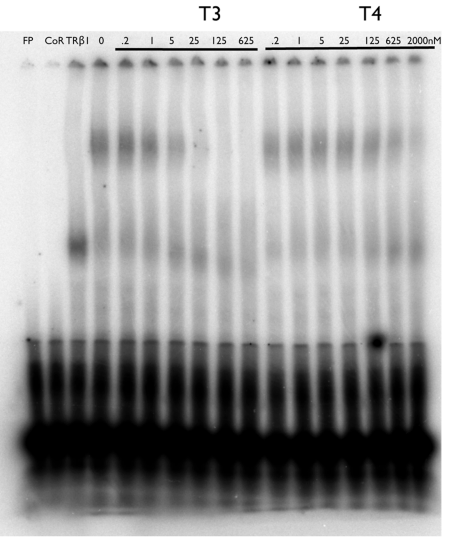
DR4:SRC1:TR $\beta$ I



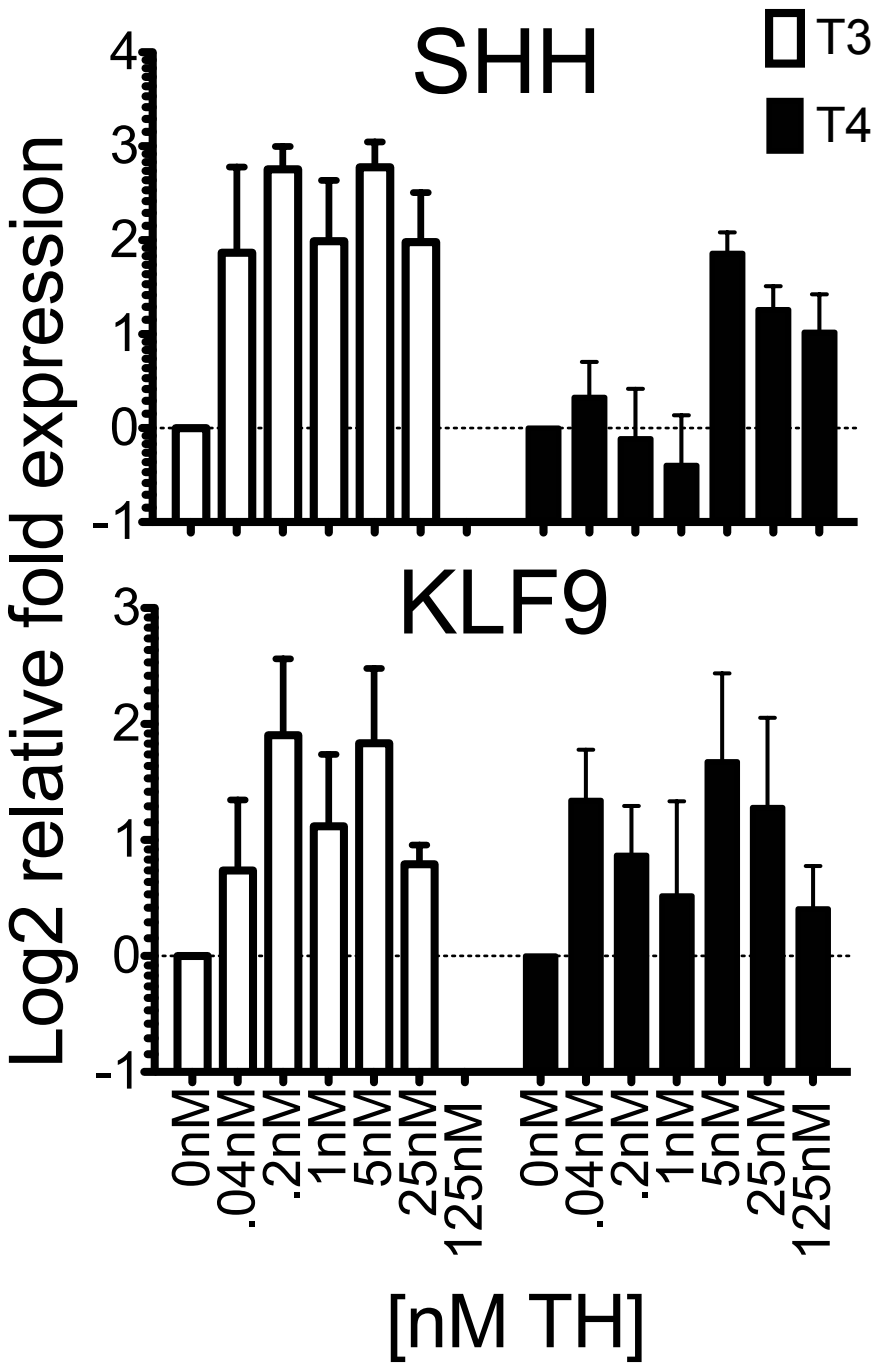
C.  
DIV6:NCoR $\omega$ :TR $\alpha$ I



DIV6:NCoR $\omega$ :TR $\beta$ I



Supplemental Figure 2



**Figure 1.**

Elastase		[nM T3]	[nM T4]	Fold change
<b>A. TR<math>\alpha</math>1</b>	EC50	0.5	2.4	4.4
	95% CI	0.3083 to 0.9333	1.228 to 4.608	
<b>B. TR<math>\beta</math>1</b>	EC50	1.0	19.8	19.0
	95% CI	0.6160 to 1.757	12.61 to 30.97	

**Figure 3.**

DR4-CON-tk-luciferase		[nM T3]	[nM T4]	Fold change
TR $\alpha$ 1	EC50	0.3	21.2	77.6
CV1	95% CI	0.1378 to 0.8202	8.441 to 44.66	
TR $\beta$ 1	EC50	1.2	49.0	40.6
CV1	95% CI	0.7470 to 1.950	30.38 to 78.90	
TR $\alpha$ 1	EC50	0.2	10.3	52.9
HEK	95% CI	0.04587 to 0.4232	2.632 to 16.65	
TR $\beta$ 1	EC50	0.6	19.1	33.1
HEK	95% CI	0.2783 to 1.191	9.437 to 38.47	
TR $\alpha$ 1	EC50	0.1	18.4	125.4
HepG2	95% CI	0.07420 to 0.2913	11.48 to 29.57	
TR $\beta$ 1	EC50	0.3	21.7	78.7
HepG2	95% CI	0.1529 to 0.4982	11.20 to 42.13	

**Figure 4.**

DR4-CON-tk-luciferase		[nM T3]	[nM T4]	Fold change
TR $\alpha$ 1	EC50	1.8	38.5	21.7
3T3L1	95% CI	0.9171 to 3.426	19.31 to 76.65	
TR $\beta$ 1	EC50	4.2	471.5	112.4
3T3L1	95% CI	2.177 to 8.081	213.8 to 1040	
TR $\alpha$ 1	EC50	1.9	33.0	17.8
HeLa	95% CI	0.8546 to 4.008	19.16 to 56.79	
TR $\beta$ 1	EC50	2.6	99.1	38.1
HeLa	95% CI	1.246 to 5.424	55.82 to 175.9	
TR $\alpha$ 1	EC50	3.4	37.1	10.9
CHO	95% CI	2.106 to 5.519	23.81 to 57.77	
TR $\beta$ 1	EC50	6.2	135.2	21.8
CHO	95% CI	2.857 to 13.49	64.42 to 283.8	

**Figure 5.**

<b>A. CHO cell transfections</b>		[nM T3]	[nM T4]	Fold change
TR $\alpha$ 1	EC50	5.8	48.5	8.4
DR4-M-tk-luciferase	95% CI	3.363 to 9.952	38.61 to 60.96	
TR $\beta$ 1	EC50	13.0	117.8	9.0
DR4-M-tk-luciferase	95% CI	8.542 to 19.91	71.27 to 194.8	
TR $\alpha$ 1	EC50	2.9	38.0	12.9
DR4- $\alpha$ MHC-tk-luciferase	95% CI	2.021 to 4.294	25.39 to 56.99	
TR $\beta$ 1	EC50	3.5	108.1	31.2
DR4- $\alpha$ MHC-tk-luciferase	95% CI	1.850 to 6.481	45.66 to 256.1	
TR $\alpha$ 1	EC50	4.9	51.4	10.5
DR4-F2-tk-luciferase	95% CI	3.190 to 7.512	32.78 to 80.66	
TR $\beta$ 1	EC50	14.0	164.7	11.8
DR4-F2-tk-luciferase	95% CI	8.700 to 22.41	93.26 to 290.8	
<b>B. HEK cell transfections</b>		[nM T3]	[nM T4]	Fold change
TR $\alpha$ 1	EC50	0.1	5.3	74.9
DR4-M-tk-luciferase	95% CI	0.02840 to 0.1744	2.655 to 10.47	
TR $\beta$ 1	EC50	0.3	34.8	116.1
DR4-M-tk-luciferase	95% CI	0.1041 to 0.8638	22.69 to 53.41	

**Figure 6.**

<b>Coactivator Pulldowns</b>		[nM T3]	[nM T4]	Fold change
TR $\alpha$ 1	EC50	12.2	24.8	2.0
SRC1	95% CI	9.569 to 15.52	19.31 to 31.89	
TR $\beta$ 1	EC50	41.8	187.6	4.5
SRC1		34.06 to 51.30	143.1 to 245.9	
TR $\alpha$ 1	EC50	14.0	9.9	0.7
TRAP	95% CI	10.44 to 18.88	7.330 to 13.48	
TR $\beta$ 1	EC50	49.5	135.6	2.7
TRAP	95% CI	40.41 to 60.67	102.2 to 180.1	
TR $\alpha$ 1	EC50	23.8	91.3	3.8
CBP	95% CI	16.35 to 34.54	58.02 to 143.7	
TR $\beta$ 1	EC50	103.4	284.5	2.8
CBP	95% CI	75.24 to 142.0	165.2 to 489.8	
TR $\alpha$ 1	EC50	10.6	61.2	5.8
GRIP	95% CI	8.546 to 13.25	44.50 to 84.19	

TRβ1	EC50	43.2	384.6	8.9
GRIP	95% CI	35.68 to 52.28	303.8 to 486.9	
TRα1	EC50	8.1	42.0	5.2
GRIP reverse	95% CI	5.914 to 11.00	30.94 to 56.87	
TRβ1	EC50	53.1	1013.0	19.1
GRIP reverse	95% CI	37.54 to 75.14	853.9 to 1202	

**Figure 7.**

<b>Mammalian-2-hybrid</b>		[nM T3]	[nM T4]	Fold change
CV-1 cells	EC50	1.3	12.0	9.2
TRα1-SRC1	95% CI	0.7-2.1	7.2-19.9	
CHO cells	EC50	9.5	71.4	7.5
TRα1-SRC1	95% CI	6.5-14.0	44.0-115.8	

**Figure 8.**

<b>coactivator EMSAs:</b>		[nM T3]	[nM T4]	Fold change
TRα1	EC50	5.0	32.9	6.6
SRC DR4	95% CI	2.918 to 8.593	18.63 to 58.18	
TRβ1	EC50	2.7	95.6	35.1
SRC DR4	95% CI	1.859 to 3.985	52.62 to 173.5	
TRα1	EC50	4.8	21.3	4.5
SRC DIV6	95% CI	3.316 to 6.817	13.52 to 33.40	
TRβ1	EC50	2.0	61.0	30.8
SRC DIV6	95% CI	1.497 to 2.618	31.02 to 120.0	
TRα1	EC50	1.7	8.4	4.8
TRAP DR4	95% CI	0.9057 to 3.313	3.912 to 17.96	
TRβ1	EC50	3.1	66.9	21.7
TRAP DR4	95% CI	2.018 to 4.715	43.18 to 103.6	
TRα1	EC50	1.9	4.0	2.2
TRAP DIV6	95% CI	1.287 to 2.679	2.926 to 5.500	
TRβ1	EC50	1.4	24.9	18.0
TRAP DIV6	95% CI	0.9944 to 1.937	12.62 to 49.23	
TRα1/RXRα	EC50	1.5	3.6	2.4
TRAP DIV6	95% CI	1.020 to 2.260	2.361 to 5.568	
TRβ1/RXRα	EC50	0.6	9.2	15.1
TRAP DIV6	95% CI	0.3539 to 1.036	3.521 to 23.79	

**Figure 9.**

<b>Corepressor Pulldown</b>		[nM T3]	[nM T4]	Fold change
TR alpha	EC50	5.8	432.0	74.3
NcoR $\omega$	95% CI	3.690 to 9.159	232.7 to 802.0	
TR $\beta$ 1	EC50	20.6	389.4	18.9
NcoR $\omega$ DIV6	95% CI	11.96 to 35.36	189.9 to 798.5	
<b>Corepressor EMSAs</b>		[nM T3]	[nM T4]	Fold change
TR $\alpha$ 1	EC50	7.8	208.3	26.7
NcoR $\omega$ DIV6	95% CI	1.929 to 7.931	11.25 to 55.03	
TR $\beta$ 1	EC50	6.4	659.5	103.1
NcoR $\omega$ DIV6	95% CI	3.859 to 10.60	143.0 to 3042	
<b>Copressor Mammalian-2-hybr</b>		[nM T3]	[nM T4]	Fold change
CHO cells	EC50	9.0	86.2	9.6
TR $\alpha$ 1-NCoR $\omega$	95% CI	4.1-19.5	5.2-1436	
CV-1 cells	EC50	1.0	41.7	41.7
TR $\alpha$ 1-NCoR $\omega$	95% CI	0.7-1.5	15.8-110.2	