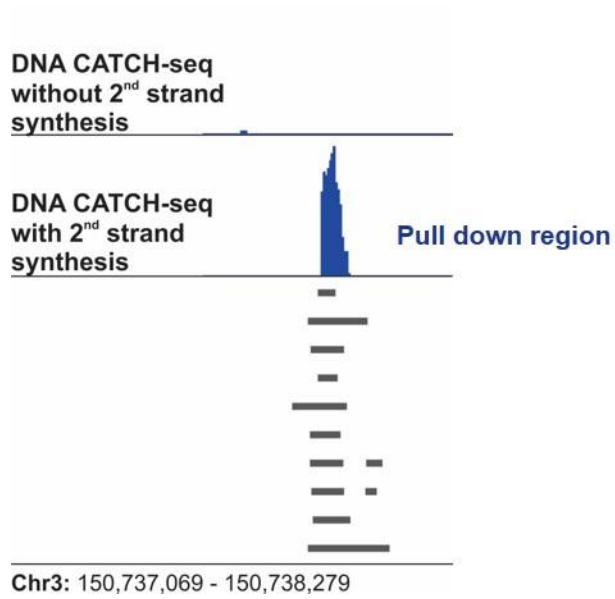
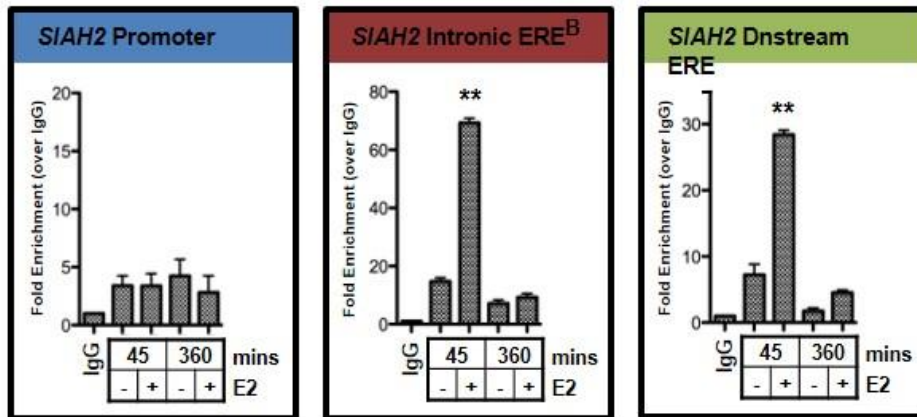
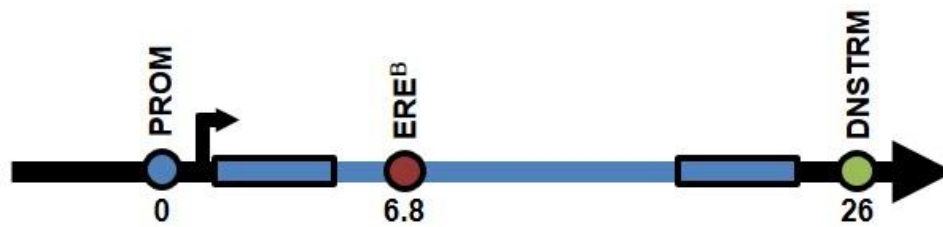


Supplementary Figure 1: (A) This diagram represents the relative location of several key EREs within and around the human *SIAH2* gene that were tested via CATCH in part B. (B) The DNA gel image shows that when specifically capturing the ERE^B locus within the intron of *SIAH2*, incomplete DNA sonication allows capture of a locus approximately 1.1 kb away (ERE^A). In addition, lack of CH₂O fixation results in capture of only the targeted fragment. However, with complete sonication and fixation, the ERE downstream of *SIAH2* is also captured with the ERE^B (DNSTRM). (C) This is a DNA gel image showing representative genomic DNA fragment size after complete sonication, using both unfixed (native) and fixed (1% CH₂O) MCF-7 cells.



Supplementary Figure 2: These histograms (same scale, Y-axis) show that CATCH-seq requires 2nd strand synthesis to visualize sequencing from the captured region (complimentary to the biotinylated oligo). The presence of the biotinylated oligo during the CATCH protocol prevents that portion of the genome from rehybridizing with its natural complimentary region of DNA. Subsequently, without second strand synthesis, this locus can not be detected by sequencing.

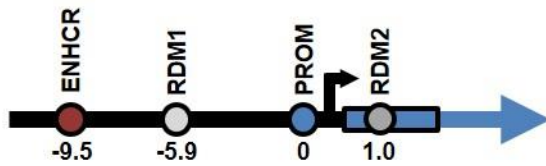
Human SIAH2



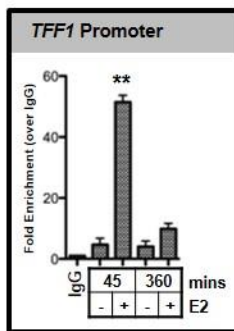
ChIP-qPCR Primers	
Oligo Name	Sequence 5' to 3'
SIAH2 PROM FWD	GGGTGTCTTTTCTGGGTIG
SIAH2 PROM REV	GTTGCCTTTCTCGTTTGAGC
SIAH2 EREB FWD	TCCACACATAACTGGCCAAA
SIAH2 EREB REV	CCAATCTGGGCTGGTATTTTA
SIAH2 DN FWD	CCTAATGGATGGCAACTGCT
SIAH2 DN REV	TTCAGATCAAAGGCCGACTC

Supplementary Figure 3: This is a chromatin immunoprecipitation experiment showing ER α binding at various locations around the *SIAH2* gene before and after E2 treatment. This experiment validated previous work showing ER α binding at the intronic and downstream EREs, as well as showing a lack of ER α binding at the *SIAH2* promoter in MCF-7 cells, which corroborates previous findings.

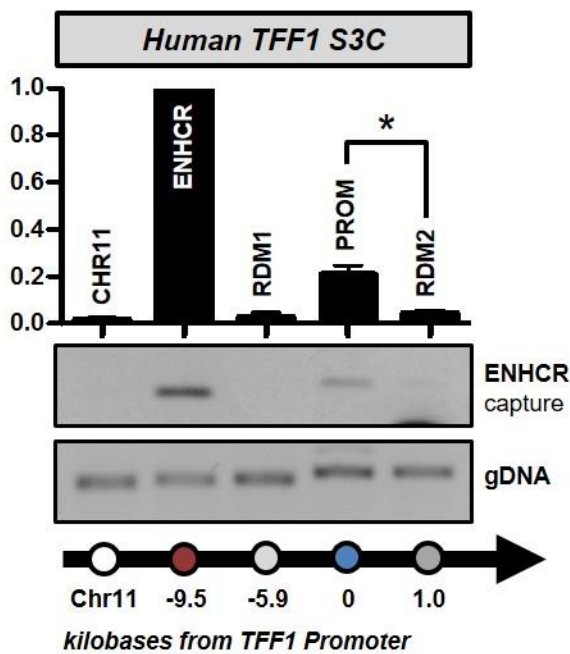
A *Human TFF1*



B

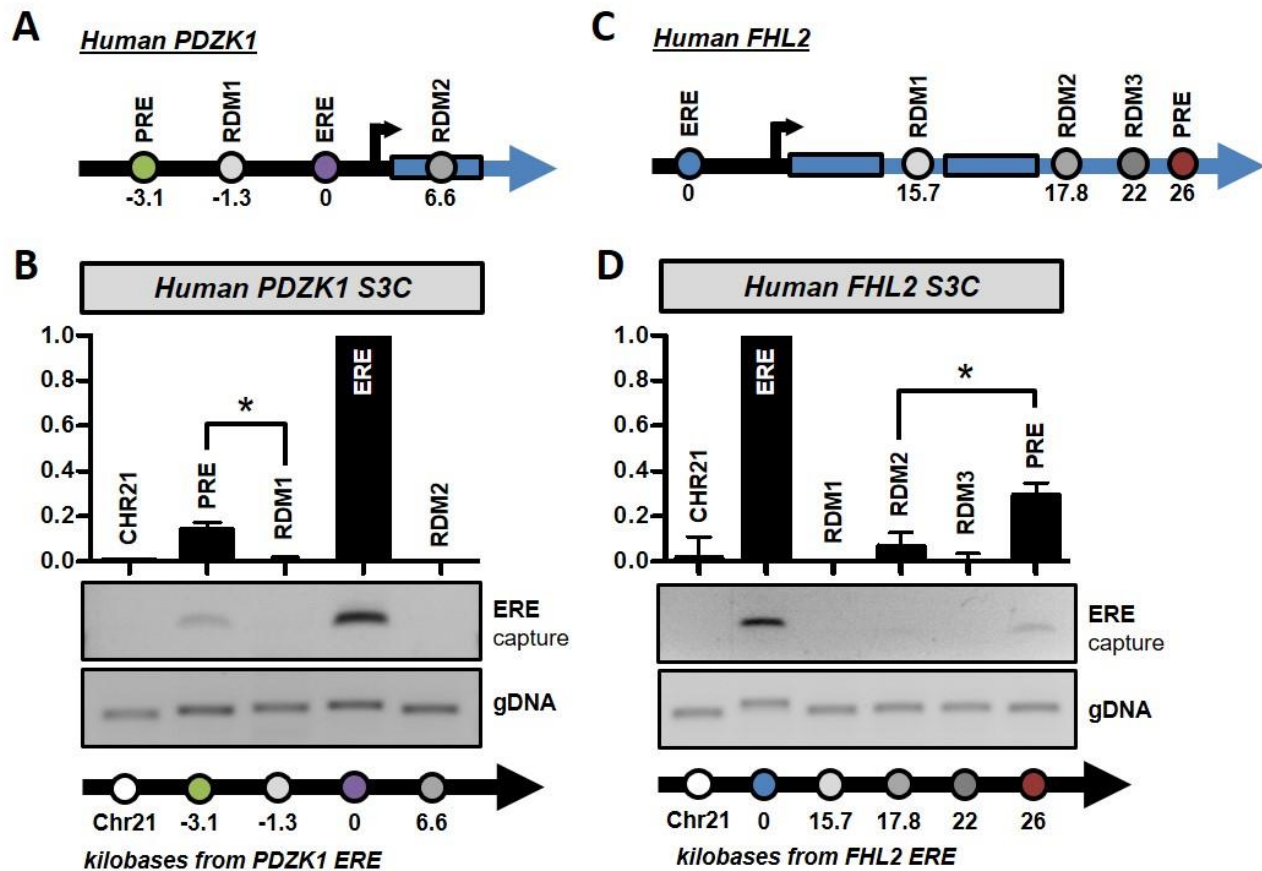


C



PCR Primers for S3C Product Detection (TFF1)			Oligos for Targeted Enrichment		
Oligo Name	Sequence 5' to 3'	PCR Product Size	Internal Oligo	bp from DNA Target	
CHR11-F	CACACACACACTATGCCTCA	100	n/a	n/a	
CHR11-R	TTCGACTCTGCGCTACCGTT				
TFF1 ENHCR-F	CITTCGGAGGGCCAGATTCA	105	/5BioTinTEG/GGTCCCCACAAAGTCAGAATTG	184	
TFF1 ENHCR-R	GACTCATGCTGCTCCCTC				
TFF1 RDM1-F	GAACTCAGAGCTCCAAGGGT	108	n/a	n/a	
TFF1 RDM1-R	GGACAAGTAGCCACCTGAGT				
TFF1 PROM-F	TGGCAAGCTACATGGAAGGA	125	n/a	n/a	
TFF1 PROM-R	AAGGTCAGGTGGAGGAGAC				
TFF1 RDM2-F	GGCAGCCGTTGATCCATTC	125	n/a	n/a	
TFF1 RDM2-R	GGTGTGTCAAGTGGATCGG				

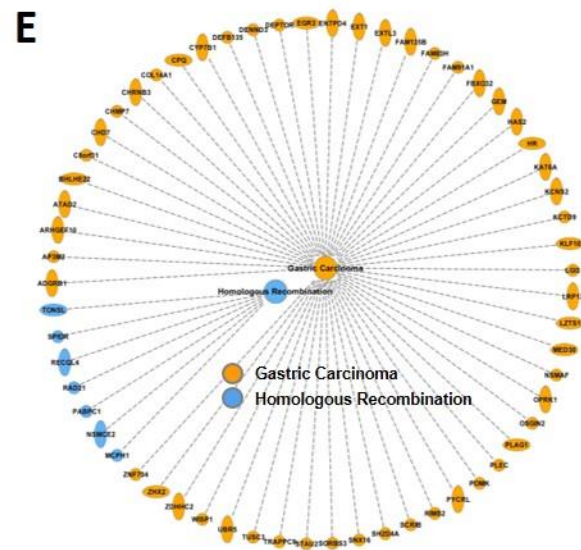
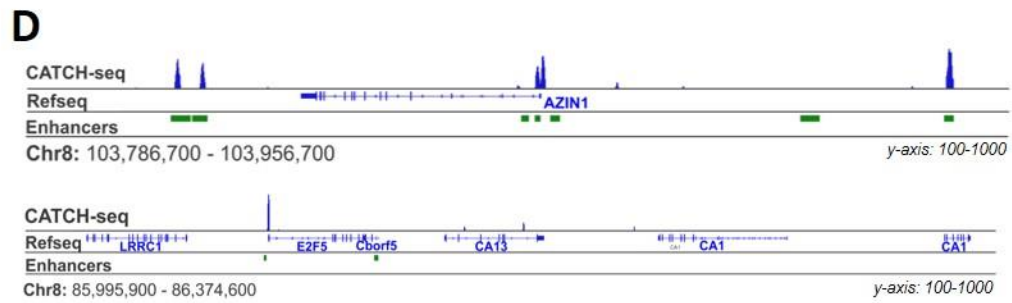
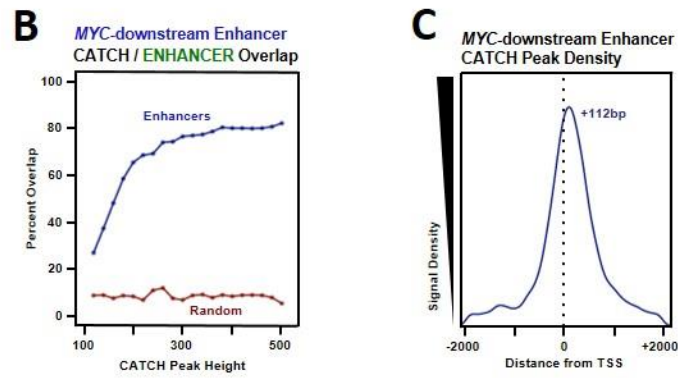
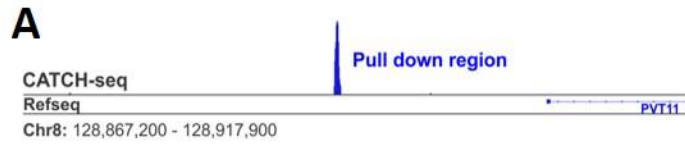
Supplementary Figure 4: **(A)** Human *TFF1* is known to have an ER α -binding enhancer region approximately 9.5 kb upstream of its TSS. This diagram shows the approximate location of that enhancer (red circle), the promoter region (blue circle), and each “random” site (grey circles) tested via CATCH, relative to each other and relative to the *TFF1* gene (blue line). **(B)** CHIP experiment showing the responsiveness of the TFF1 promoter to E2 treatment at 45 minutes. Served to validate previous findings, as well as positive control for E2-responsiveness in Supplemental Figure S3. **(C)** The gel image shows the relative intensity of PCR products associated with each tested locus around *TFF1*. The gDNA gel represents the total genomic input to the CATCH protocol, while the ENHCR capture gel represents just the pool of DNA that was enriched after CATCH was done targeting the enhancer region upstream of *TFF1*. The graph displays the mean (SEM error bars) of three experimental replicates. Student’s unpaired t-test of data sets with unequal variance was performed to compare the strongest negative control signal against the promoter region (* denotes $p \leq 0.05$).



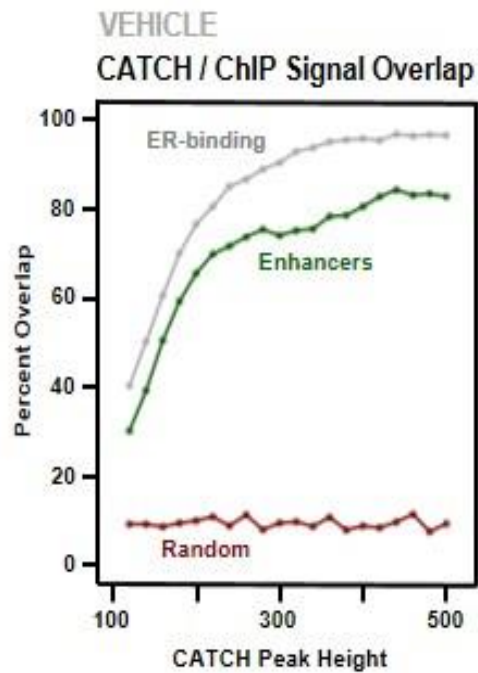
PCR Primers for S3C Product Detection (PDZK1)			Oligos for Targeted Enrichment	
Oligo Name	Sequence 5' to 3'	PCR Product Size	Internal Oligo	bp from DNA Target
CHR21-F	GGCTTGTTTGAGAGAGCGAG	100	n/a	n/a
CHR21-R	TTAACATTGCCCTTGTGCC			
PDZK1 PRE-F	CAGAGTACACAGTCGCCTCT	111	n/a	n/a
PDZK1 PRE-R	GCTCCAGTGGTTTTCTCTCC			
PDZK1 RDM1-F	TGGACTCAAGCATTCTCTCC	118	n/a	n/a
PDZK1 RDM1-R	AGCAATCTGGTCAGGAAGCT			
PDZK1 ERE-F	GGGATTGCGATGAAGTCTCAGG	123	/5BioTintEG/TGAAAGATATAGAGGAGGCCAGGAG	158
PDZK1 ERE-R	ACCAGCTTATCTCTCCACC			
PDZK1 RDM2-F	TGCAGGGATTACAGGTGTGA	114	n/a	n/a
PDZK1 RDM2-R	CCCCTGACATTCTAGCTTGGGA			

PCR Primers for S3C Product Detection (FHL2)			Oligos for Targeted Enrichment	
Oligo Name	Sequence 5' to 3'	PCR Product Size	Internal Oligo	bp from DNA Target
CHR21-F	GGCTTGTTTGAGAGAGCGAG	100	n/a	n/a
CHR21-R	TTAACATTGCCCTTGTGCC			
FHL2 ERE-F	GCTAGCCCACCAGCCTC	129	/5BioTintEG/CTAGAAGCCCTGCCTTTCTTTGG	175
FHL2 ERE-R	GGGTCTGAGCTGTACAAATGC			
FHL2 RDM1-F	ACACCAGCTATTCTGTGGT	110	n/a	n/a
FHL2 RDM1-R	CGCAGTGTGAATAAGCAGCA			
FHL2 RDM2-F	CCCTGATCCACCACTGAAGT	109	n/a	n/a
FHL2 RDM2-R	GTGGCAGAGATCACATTCTG			
FHL2 RDM3-F	AAACCCACCCTTCTGTCTCTC	105	n/a	n/a
FHL2 RDM3-R	GCTGGACCCTGAGAAATGTGA			
FHL2 PRE-F	CTGAGAGAAACGTTGCGGAG	108	n/a	n/a
FHL2 PRE-R	CAAAAGACAGAGTGCCTTCCA			

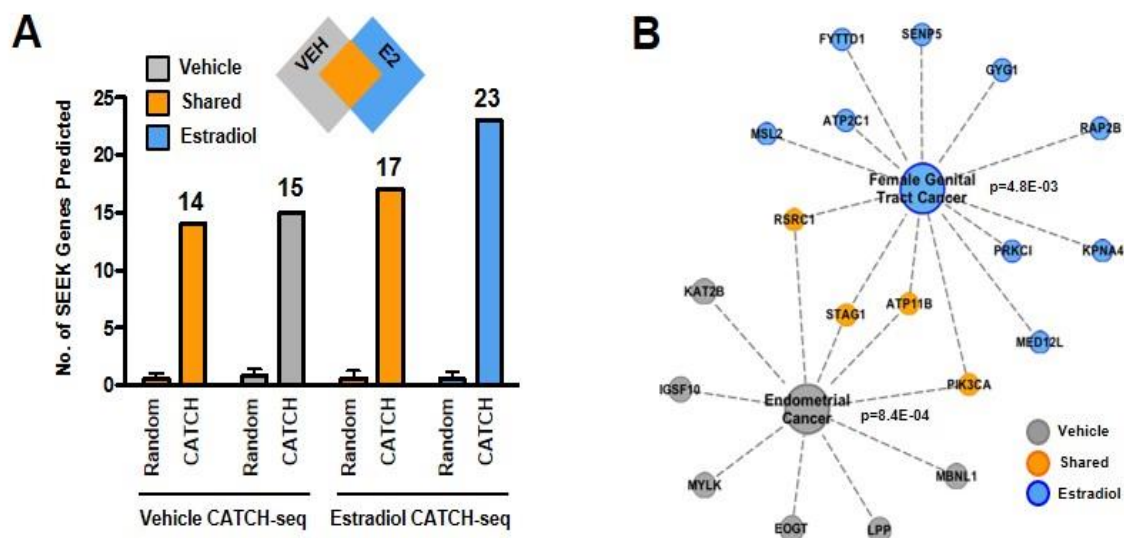
Supplementary Figure 5: (A) Human *PDZK1* contains both ER and PR binding sites just upstream of the TSS. This diagram shows the approximate location of both of those sites (PRE: green and ERE: purple), as well as each “random” site (grey circles) tested via CATCH in part B. **(B)** The gel image shows the relative intensity of PCR products associated with each tested locus around *PDZK1*. The gDNA gel represents total genomic input, and the ERE capture visualizes only the pool of DNA that was enriched after CATCH targeting the estrogen binding element just upstream of the *PDZK1* TSS. The graph displays the mean (SEM error bars) of three experimental replicates. Student’s unpaired t-test of data sets with unequal variance was performed to compare the strongest negative control signal against that of the PRE (* denotes $p \leq 0.05$). **(C)** The human *FHL2* gene has an ERE located about 5 kb upstream, and a PRE about 21 kb downstream within its second intron. This diagram shows the relative position of the ERE (blue circle), PRE (red circle), and random sites (grey circles) tested in part D. **(D)** The gel image shows the relative intensity of PCR products associated with each tested locus around *FHL2*. The gDNA gel represents total genomic input, and the ERE capture visualizes only the pool of DNA that was enriched after CATCH targeting the estrogen binding element upstream of the *FHL2* TSS. The graph displays the mean (SEM error bars) of three experimental replicates. Student’s unpaired t-test of data sets with unequal variance was performed to compare the strongest negative control signal against that of the PRE (* denotes $p \leq 0.05$).



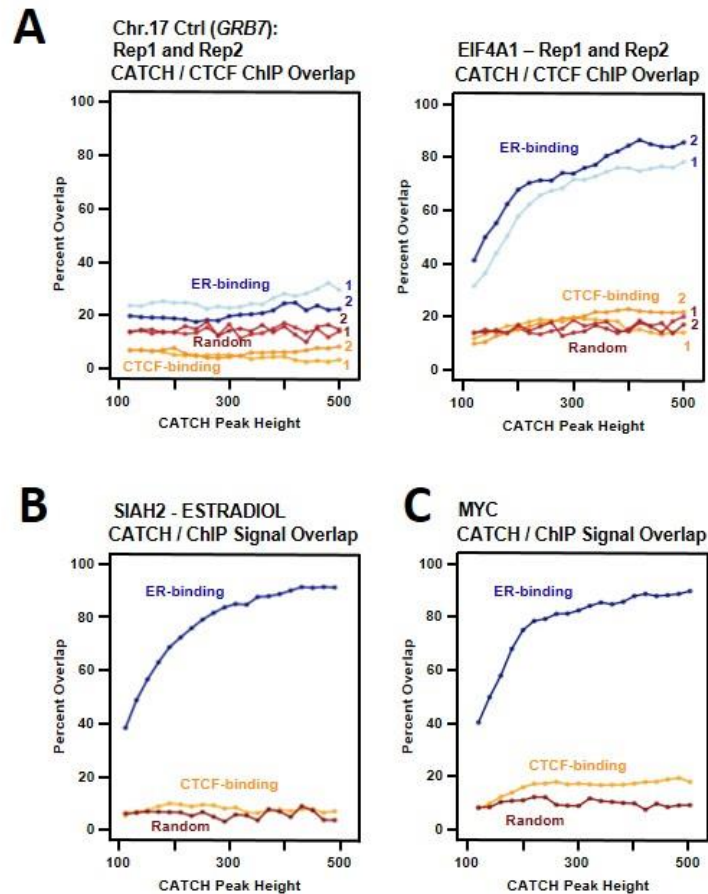
Supplementary Figure 6: (A) An enhancer region downstream of *MYC* was directly captured via CATCH. (B) Enhancer downstream of *MYC* CATCH-seq interactions significantly overlap with enhancer marks (blue) compared to statistically randomized control (red); the frequency of these overlaps increase with peak height. (C) The CATCH-seq interactions of the enhancer downstream of *MYC* occur, on average, 112 bp downstream of TSS's on chromosome 8. (D) Examples of raw CATCH-seq histograms (background subtracted) demonstrating the locations of peaks overlapping with enhancer marks. Additionally, the peak at the *E2F5* promoter demonstrates the specificity of CATCH, as no other gene promoters in the region are positive for long-distance interactions. (E) IPA analysis detailing specific gene promoters that demonstrated physical interaction with the enhancer downstream of *MYC*. The most significantly enriched process (blue) and disease (orange) enriched within this dataset was homologous recombination and gastric carcinoma, respectively.



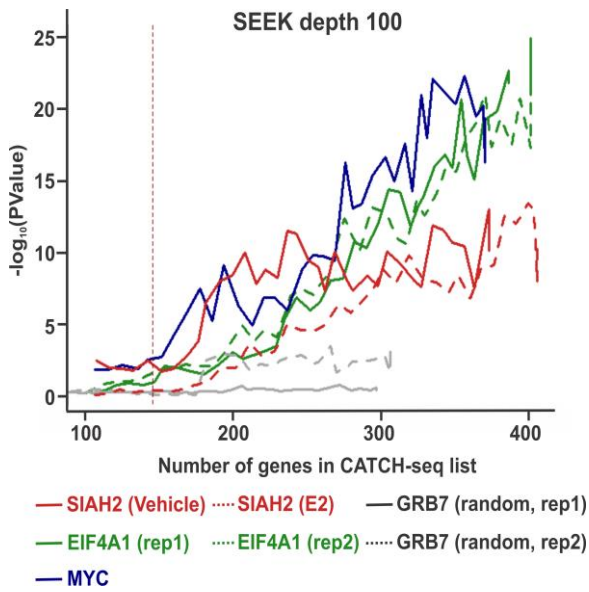
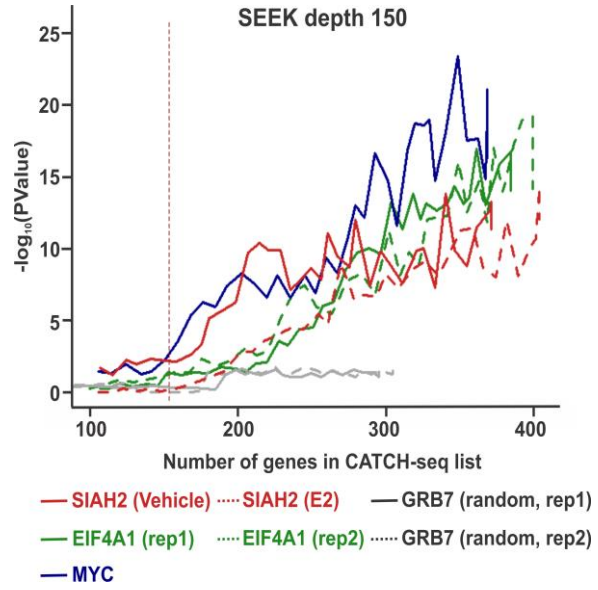
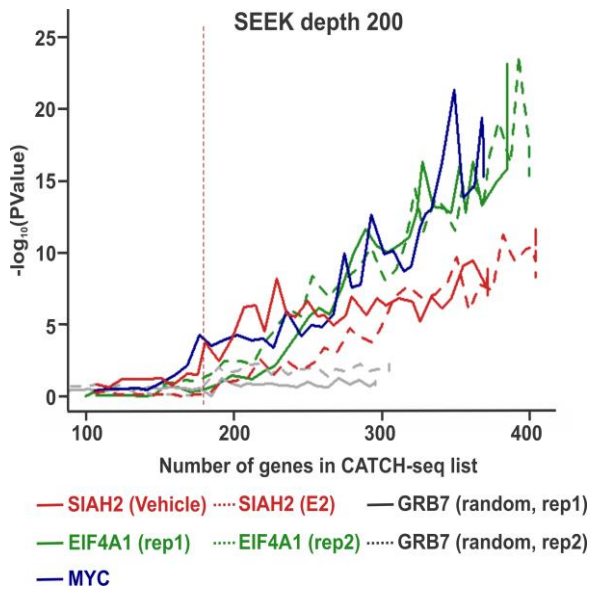
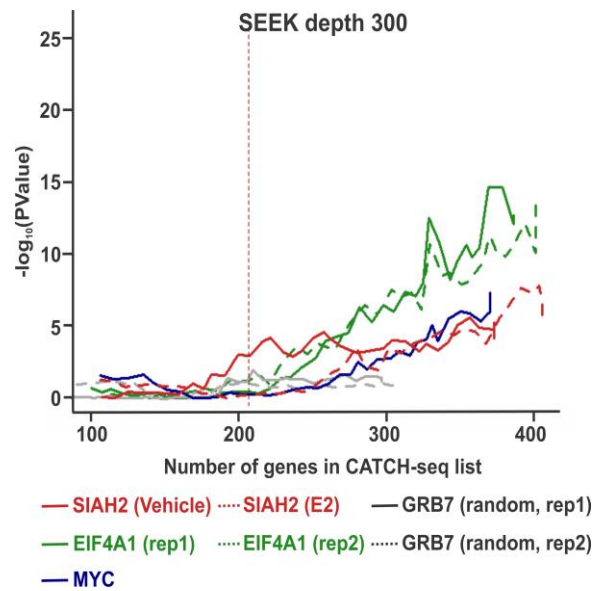
Supplementary Figure 7: *SIAH2* (vehicle-treated) enhancer CATCH-seq interaction peaks significantly overlap with enhancer marks (green) and ER-binding sites (gray) compared to statistically random control (red); the frequency of these overlaps increase with peak height. The same pattern is observed with estradiol treatment in Figure 4E.



Supplementary Figure 8: (A) Graph detailing the number of SEEK genes ‘predicted’ by the vehicle-treated (gray) or estradiol-treated versions of *SIAH2* enhancer CATCH-seq genome-wide. Genes predicted by both conditions (the majority) are in orange. **(B)** IPA showing the topmost statistically significant and enriched pathway for each small gene subset identified by CATCH-seq / SEEK overlap: female genital tract cancer (estradiol treated; blue) and endometrial cancer (vehicle treated; gray).



Supplementary Figure 9: Analysis of CTCF binding adjacency/overlap with CATCH peaks. Numbers 1 and 2 denote replicates for *GRB7* and *EIF4A1*. **(A, left)** Control *GRB7* promoter region CATCH-seq interaction peaks do not significantly overlap with CTCF-binding sites (orange) and have very modest overlap with ER-binding sites (blue) compared to statistically random control (red); the frequency of these overlaps does not increase with peak height. **(A, right)** *EIF4A1* promoter region CATCH-seq interaction peaks do not significantly overlap with CTCF-binding sites (orange), but have significant overlap with ER-binding sites (blue) compared to statistically random control (red); the frequency of the latter overlaps increases as a function of peak height. **(B)** *SIAH2* downstream enhancer region CATCH-seq interaction peaks do not significantly overlap with CTCF-binding sites (orange), but have significant overlap with ER-binding sites (blue) compared to statistically random control (red); the frequency of the latter overlaps increases as a function of peak height. **(C)** *MYC* downstream enhancer region CATCH-seq interaction peaks show modest overlap with CTCF-binding sites (orange), but have significant overlap with ER-binding sites (blue) compared to statistically random control (red); the frequency of both overlaps increases, to some degree, as a function of peak height.

A**B****C****D**

Supplementary Figure 10: The graphs depict, using a constant SEEK list of the top 100, 150, 200, or 300 identified genes, the continuous p-value of each CATCH experiment as the number of CATCH genes identified (used) in the analysis decreases. The vertical dotted red lines indicate the average point (of all CATCH experiments on the graph) at which CATCH results become non-significant ($p=0.01$). As CATCH is used to identify more gene promoters, its ability to predict transcriptionally co-expressed genes (as measured by SEEK) increases. The negative \log_{10} of the p value was plotted on the y-axis against the number of CATCH genes used on the x-axis. The continuous p value was graphed for every CATCH experiment at varying steady-state SEEK depths of 100 (A), 150 (B), 200 (C) and 300 (D). The p-value here, is the effective point at which CATCH results can no longer, with p degree of certainty, be sure that a given identified gene is reliably co-expressed.

Supplementary Table 1. Basic Comparison of DNA-DNA Interaction Detection Techniques

Technique	Cardinality	Resolution	Advantages	Disadvantages
3C-qPCR	One-to-one	kilobases	Simple	Long protocol, labor intensive, many controls, foreknowledge of interactions required
3C/4C-seq	One-to-all	kilobases	Improved resolution over qPCR, can adapt for higher throughput	Single locus per experiment, more complex data analysis
5C	Many-to-many	kilobases	Can identify interactions between many fragments	Highly labor intensive, not genome-wide, challenging primer design, more complex data analysis
Hi-C	All-to-all	kilo/megabases	Genome-wide, all-to-all interactions	Cost-prohibitive, massive sequencing depth required, poor resolution, advanced bioinformatics required, all-to-all can be confounding
ChIA-PET	Many-to-all	kilobases	Genome-wide detection, enzymatic digestion not required	Requires antibodies, can only detect the interactions resulting from a single protein
T2C	Many-to-all	kilobases	Can detect regional interactions, less expensive than Hi-C or 5C	Restricted to selected genomic regions, advanced bioinformatics required, large primer design effort
CATCH	One-to-all	centibases	Most cost effective technique, takes only 24 hours to complete, no enzymatic digestion/ligation, highest resolution/detection	Requires selecting locus to probe (one-to-all), single pulldown locus per experiment

Supplementary Table 2. SEEK list "seed" genes from associated CATCH pulldowns

Chr17 Ctrl <i>GRB7</i> 17q12		<i>EIF4A1</i> 17p13		<i>SIAH2</i> (vehicle) 3q25		<i>SIAH2</i> (estradiol) 3q25		<i>MYC</i> 8q24.21	
Gene	Cytoband	Gene	Cytoband	Gene	Cytoband	Gene	Cytoband	Gene	Cytoband
<i>ZNF385C</i>	17q21.2	<i>FXR2</i>	17p13.1	<i>GYG1</i>	3q24-25.1	<i>TSC22D2</i>	3q25.1	<i>TRIB1</i>	8q24.13
<i>KRT31</i>	17q21.2	<i>SEN3</i>	17p13	<i>IGSF10</i>	3q25.1	<i>EIF2A</i>	3q25.1	<i>FAM84B</i>	8q24.21
<i>KRT39</i>	17q21.2	<i>POLR2A</i>	17p13.1	<i>MBNL1</i>	3q25	<i>MED12L</i>	3q25.1	<i>SQLE</i>	8q24.1

Supplementary Table 3. CATCH pull-down and SEEK gene list details

CATCH pull-down Experiment	chromosomal location of pull-down	Number of total SEEK genes on chromosome	Number of SEEK genes analyzed (top hits; genome)	Number of SEEK genes analyzed from pull-down chr	Number of genes below p value 0.01	Number of SEEK genes predicted by CATCH (below p value 0.01)	Percent SEEK genes predicted
Chr17 Ctrl (<i>GRB7</i>)	chr:17q12	993	100	27	26	0	0.0%
<i>EIF4A1</i>	chr:17p13	1001	100	74	67	16	23.9%
<i>MYC</i>	chr:8q24.21	565	100	82	71	46	64.8%
<i>SIAH2</i> (vehicle)	chr:3q25	939	150	62	49	15	30.6%
<i>SIAH2</i> (estradiol)	chr:3q25	948	150	70	54	23	42.6%

Supplementary Table 4. Effect of total SEEK genes analyzed on CATCH/SEEK prediction

Sample	No. top SEEK Genes Analyzed	No. SEEK genes on pulldown chr*	Seek genes predicted at random*	SEEK genes predicted by CATCH*	p value
<i>GRB7</i> (ctrl)	100	26	1.7	0	0.12
<i>GRB7</i> (ctrl)	200	35	2.5	0	0.1
<i>GRB7</i> (ctrl)	500	48	4.4	0	0.03
<i>EIF4A1</i>	100	67	9.1	16	3.70E-03
<i>EIF4A1</i>	200	83	11.5	23	8.42E-05
<i>EIF4A1</i>	500	96	17	30	8.72E-04
<i>SIAH2</i> vehicle	150	49	4.4	15	1.80E-08
<i>SIAH2</i> vehicle	200	53	5.2	15	4.46E-05
<i>SIAH2</i> vehicle	500	59	8.5	17	3.00E-03
<i>SIAH2</i> E2	150	54	8	23	3.60E-13
<i>SIAH2</i> E2	200	58	9.8	25	4.57E-12
<i>SIAH2</i> E2	500	71	14.4	34	1.08E-08
<i>MYC</i>	100	71	20	46	2.13E-12
<i>MYC</i>	200	96	29.2	62	2.76E-16
<i>MYC</i>	500	110	37.5	72	4.06E-11

*p value cutoff 0.01

Supplementary Table 5. CATCH-SEEK Gene-expression Prediction					
SIAH2 Enhancer CATCH / Vehicle			SIAH2 Enhancer CATCH / Estradiol		
Gene	Co-exp Value	p value	Gene	Co-exp Value	p value
<i>AADACP1</i>	---	---	<i>TSC22D2</i>	---	---
<i>IGSF10</i>	---	---	<i>EIF2A</i>	---	---
<i>MBNL1</i>	---	---	<i>MED12L</i>	---	---
<i>MFSD1</i>	1.1327	0	<i>KPNA4</i>	1.004	0.0001
<i>ARL6IP5</i>	0.9526	0.0026	<i>STAG1</i>	0.9164	0.0008
<i>ACAP2</i>	0.9442	0.0041	<i>CCNL1</i>	0.8872	0.0014
<i>KAT2B</i>	0.8731	0.0006	<i>ACAP2</i>	0.8624	0.0053
<i>PLSCR4</i>	0.8729	0.0059	<i>FYTTD1</i>	0.8315	0.0026
<i>PIK3CA</i>	0.8722	0.0081	<i>PIK3CA</i>	0.8125	0.0062
<i>STAG1</i>	0.831	0.0082	<i>DLG1</i>	0.7784	0.0017
<i>RAB7A</i>	0.7988	0.0062	<i>TBC1D23</i>	0.7773	0.0021
<i>ZBTB38</i>	0.7949	0.0013	<i>ATP11B</i>	0.7716	0.0013
<i>EOGT</i>	0.793	0.0037	<i>PRKCI</i>	0.7609	0.0002
<i>LPP</i>	0.7367	0.0052	<i>CDV3</i>	0.7466	0.0015
<i>CDV3</i>	0.7348	0.0054	<i>ZNF639</i>	0.7423	0.0005
<i>RSRC1</i>	0.7215	0.0018	<i>CLDND1</i>	0.7336	0.0014
<i>ATP11B</i>	0.7129	0.0096	<i>RSRC1</i>	0.7299	0.0003
<i>MYLK</i>	0.6905	0.0078	<i>ATP2C1</i>	0.7268	0.0071
			<i>RASA2</i>	0.7165	0.0019
			<i>SSR3</i>	0.6475	0.0031
			<i>RAP2B</i>	0.6412	0.0002
			<i>SIAH2</i>	0.6313	0.0006
			<i>LRRC58</i>	0.6276	0.0042
			<i>GYG1</i>	0.6146	0.003
			<i>SENP5</i>	0.6083	0.0049
			<i>MSL2</i>	0.608	0.0067

Supplementary Table 5: Table contains the lists of genes that had both significant CATCH-seq signal peaks within 2kb of their promoters, and were also identified by SEEK as being transcriptionally co-expressed with one another. **NB:** *SIAH2* is independently identified (w/ estradiol treatment).

Supplementary Methods

Capture of Associated Targets on Chromatin User Protocol

Part 1: Nuclear isolation, crosslinking and chromatin shearing

1. Be sure that cells are in log-phase growth.
2. Aspirate cell-growth media.
3. Add 2% Formaldehyde (in serum-free RPMI) (2 ml 16% formaldehyde per 14 ml PBS) to cell culture.
4. Incubate for 10 min at 25°C, gently rocking.
5. Aspirate 2% formaldehyde solution.
6. Quench crosslinking by adding 10 ml of 250 mM Tris-HCl pH 8.0 (in PBS).
7. Incubate for 5 min at 25°C, gently rocking.
8. Scrape cells in 1.0 ml PBS to harvest.
9. Centrifuge cells at 1,500 x g for 6 minutes and aspirate supernatant.
10. Wash cell pellet in PBS and resuspend in 500 µl Sucrose Buffer + PIC.
11. Dounce homogenize 30 times with a tight fitting pestle.
12. Centrifuge for 10 minutes at 6500 RPM at 4°C.
13. Aspirate supernatant and resuspend pellet again in 120 µl of CATCH buffer + PIC.
14. Sonicate samples (BioRuptor, 2 x 7 minutes on High, 30s on/off).
15. Pellet debris at 14,000 RPM for 10 minutes at 4°C.
16. Transfer supernatant to a new 1.5 ml tube.

Part 2: Pre-clearing and hybridization

1. Incubate the chromatin sample at 58°C for 5 minutes to unmask endogenous biotin.
2. Add 10 µl of pre-equilibrated (in CATCH buffer) streptavidin magnetic beads to the sample.
3. Incubate 60 min at 25°C while rotating.
4. Pull out magnetic beads and transfer cleared chromatin supernatant to a PCR tube.
5. Add biotinylated oligo probe (300 nM final conc.) to the chromatin sample.
6. Hybridize the probe to the chromatin as follows (**blue** step should be at least 5°C above the T_m of the oligo; **red** step should be done below the T_m of the oligo):
 - 25°C for 1 min (equilibration)
 - 81°C for 4 min (gDNA denaturation)
 - 72°C - 50°C** decreasing gradient (15 sec per 1°C)
 - 42°C** for 10 min (hybridization)
 - 25°C hold
7. Remove unhybridized oligo and other impurities with illustra sephacryl (S-400 HR) microspin column according to manufacturer's instructions.
8. Transfer sample to a new 1.5 ml tube and add 300 µl of DNase/RNase free H₂O.
9. Add 25 µl of pre-equilibrated (in CATCH buffer) streptavidin magnetic beads to the sample.
10. Incubate for 60 min at 25°C while rotating.

Part 3: De-crosslinking and protein digestion

1. Immobilize beads on magnetic stand, aspirate unhybridized supernatant and wash samples 3x in CATCH buffer at 42°C while shaking at 1000 rpm on a thermomixer.
 2. Resuspend beads in 150 µl of De-crosslinking buffer supplemented with 1 µl of 20 mg/ml RNase A.
 3. Incubate at 55°C while shaking at 700 rpm on a thermomixer for 60 minutes.
 4. Add 5 µl of 20 mg/ml Proteinase K to each sample.
- USER PROTOCOL
5. Incubate at 65°C while shaking at 700 rpm on a thermomixer for 3-6 hr (or overnight) to de-crosslink.

6. Incubate at 100°C for 1 minute.
7. Remove magnetic beads and transfer DNA sample to a new 1.5 ml tube.

Part 4: DNA Purification

1. Extract once with Phenol:Chloroform:IAA.
2. Extract again with Phenol:Chloroform:IAA, and then once with Chloroform to remove any residual Phenol.
3. Add 10 µg glycogen, 1/10 volume of 3M Sodium Acetate pH 5.2, and 2.5 volumes of 200 proof ethanol.
4. Precipitate DNA at -20°C or at -80°C for 2 hours.
5. Centrifuge at 14,000 rpm for 30 minutes, wash with 70% ethanol and dry pellet.
6. Resuspend the DNA pellet in 30 µl of 10mM Tris-Cl pH 8.5 or nuclease-free H₂O.
7. Proceed immediately to 2nd strand synthesis.

CATCH Buffer (40 ml)

- 400 µl of 1.0 M HEPES (10 mM)
- 240 µl of 5.0 M NaCl (30 mM)
- 160 µl of 0.5 M EDTA pH 8.0 (2 mM)
- 400 µl of 20% SDS (0.2%)
- H₂O to 40 ml
- Add Protease Inhibitors fresh

De-crosslinking Buffer (10 ml)

- 300 µl of 1.0 M Tris-Cl pH 8.0 (30 mM)
- 60 µl of 5.0 M NaCl (30 mM)
- 10 µl of 0.5 M EDTA (0.5 mM)
- 2 µl of 0.5 M EGTA (0.1 mM)
- 500 µl of 20% (w/v) SDS (1%)
- H₂O to 10 ml
- Add RNase and Proteinase K (fresh)

Sucrose Buffer (10 ml)

- 100 µl of 1.0 M HEPES pH 8.0 (10 mM)
- 1.03 g of Sucrose (0.3 M)
- 30 µl of 1.0 M CaCl₂ (3 mM)
- 20 µl of 1.0 M MgOAc (2 mM)
- 100 µl of Triton-X100 (1%)
- H₂O to 10 ml