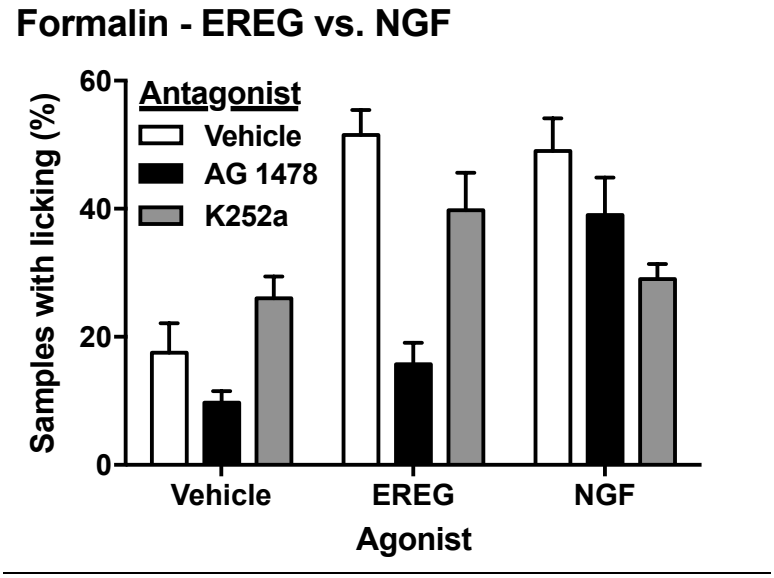
Supplemental Methods

Drosophila experiments

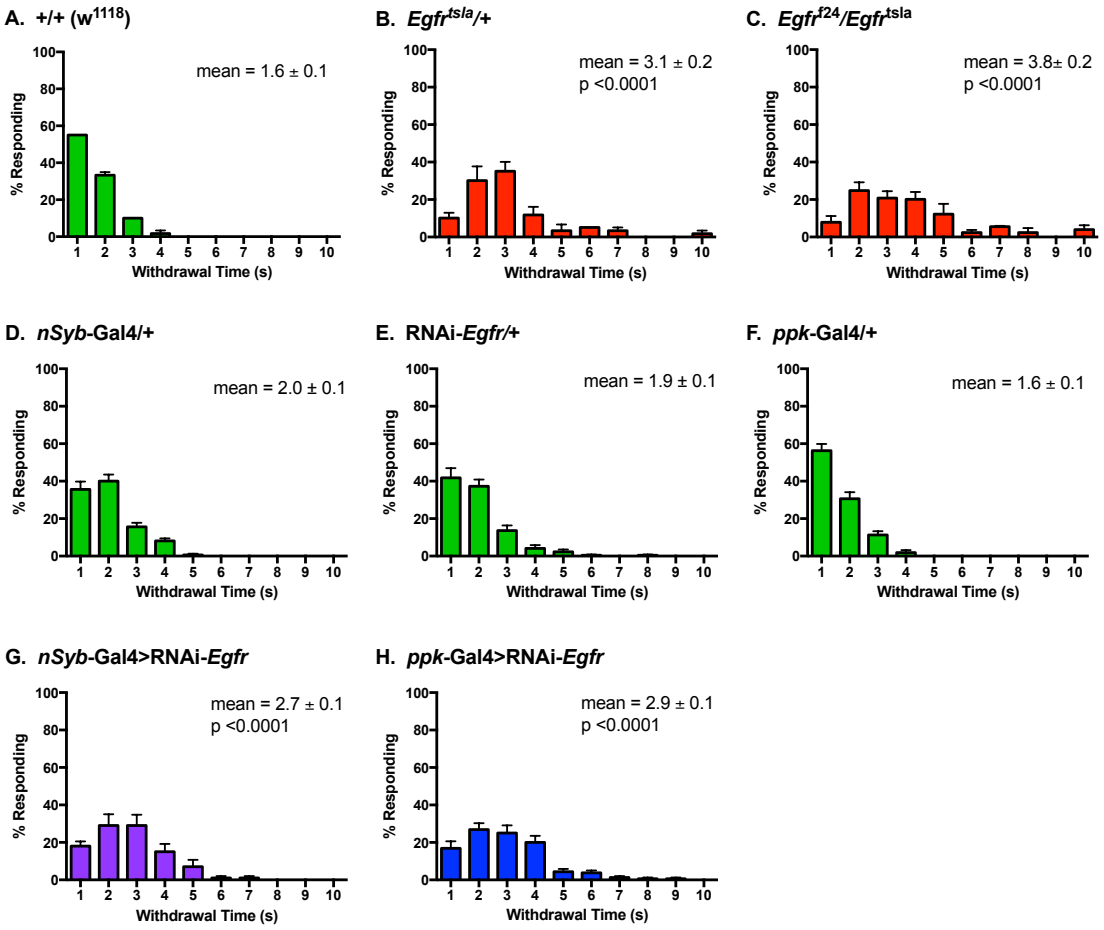
Flies were reared on cornmeal-molasses-yeast agar at 25 °C, 70% humidity, on a 12:12-h light/dark cycle. *ppk-Gal4, Egfr* mutants (*Egfr*^{f24}, *Egfr*^{tsla}), and the UAS-*Egfr* rescue line were obtained from the Bloomington Drosophila Stock Centre (BDSC; Bloomington, IL). *Neuronal Synaptobrevin-GAL4* (*nSyb-Gal4*) was obtained from Julie Simpson (Janelia Farm Research Campus, VA). Wildtype *w*^{1118} and *Egfr* short hairpin RNA-interference (RNAi) (transformant ID 107130) flies were obtained from the VDRC (Vienna, Austria).

To assess nociceptive behavior, third instar larvae were transferred to a 100 mm petri dish containing a thin film of distilled water and allowed a 10-min rest period. After this time, they were touched on abdominal segments A4-A6 with a heat probe consisting of a sharpened soldering iron with the tip heated to 46 °C. The response time was recorded as the time elapsed between application of the heat probe and the elicitation of the characteristic nociceptive withdrawal response, a 360° rolling motion about the lateral axis.

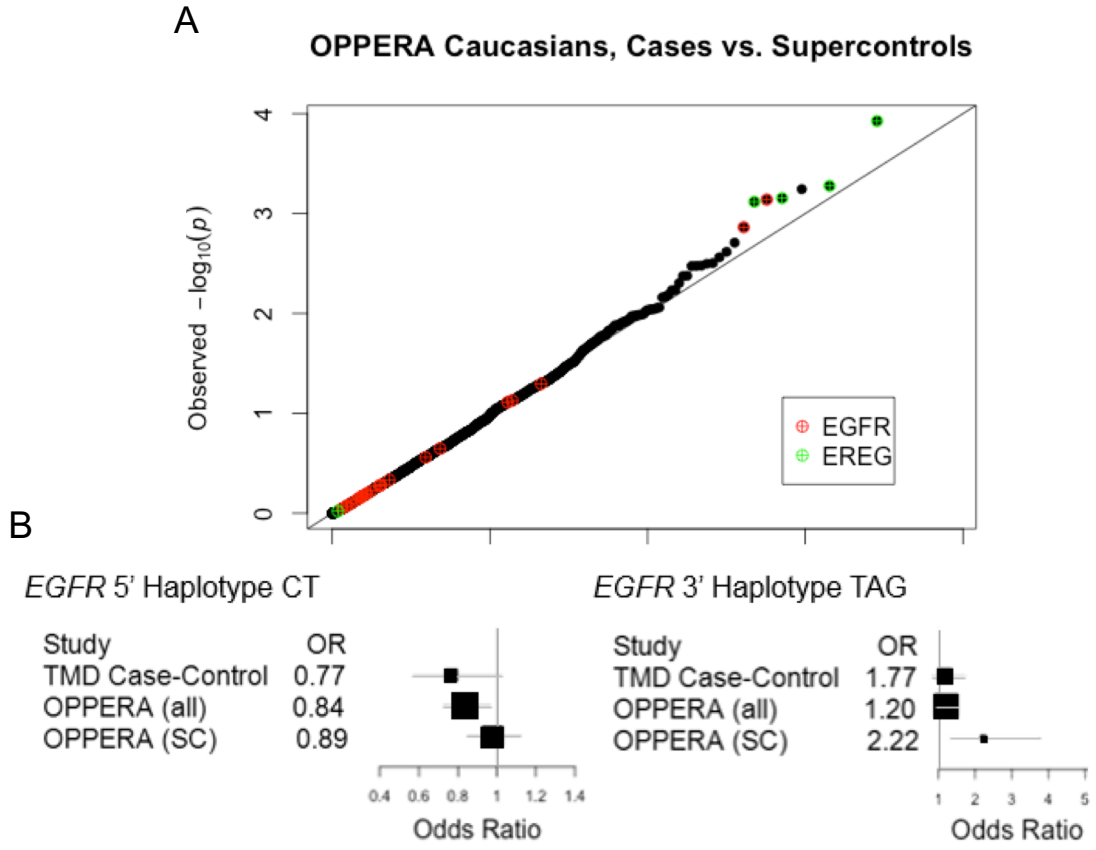
Supplemental Figures.



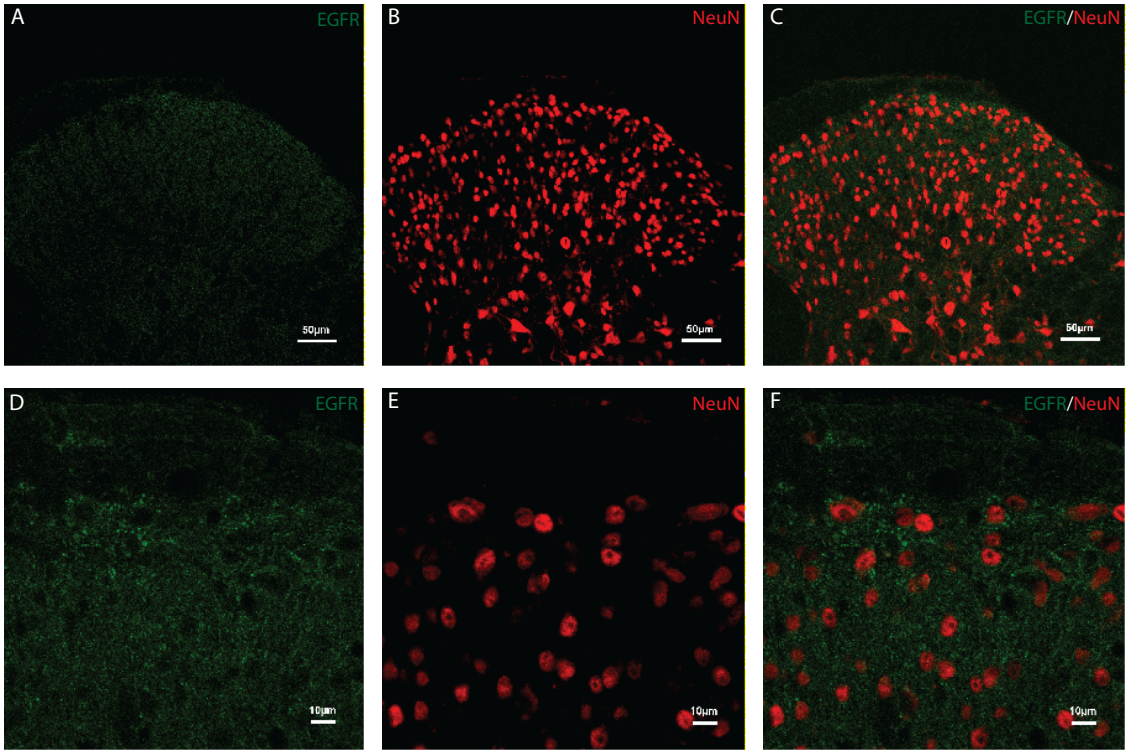
Supplemental Figure 1. The EGFR inhibitor AG 1478, but not the Trk blocker, K252a, prevents EREG-induced hypersensitivity on the formalin test. Conversely, K252a, but not AG 1478, blocks NGF-induced hypersensitivity. Agonist x antagonist interaction: $F_{4,59}$=5.4, p=0.001. Bars represent mean±SEM percentage of samples featuring licking/biting behavior; n=6–8/drug/dose.



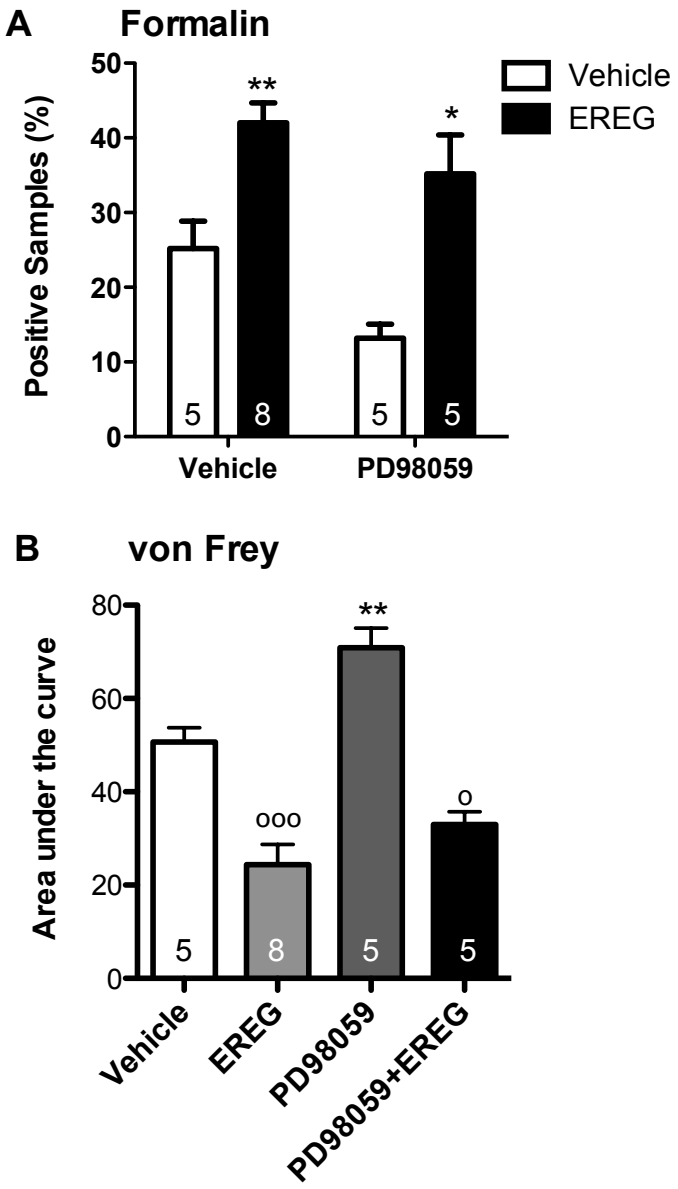
<u>Supplemental Figure 2.</u> *Egfr* knockdown alters nociceptive responses to noxious thermal stimuli in *Drosophila*. While homozygous *Egfr* mutations are lethal, heterozygous and trans-heterozygous mutants displayed a strong analgesic phenotype in response to a 46 °C probe (Kruskal-Wallis statistic = 62.6, $p<0.0001$) (**A–C**). Using pan-neuronal RNAi knockdown (*nSyb*-Gal4), EGFR was found to be acting in the nervous system (Kruskal-Wallis statistic = 42.0, $p<0.0001$) (**D, E, G**), and a requirement for EGFR was further traced down to class IV sensory neurons using *ppk*-Gal4 (Kruskal-Wallis statistic = 92.2, $p<0.0001$) (**E, F, H**).



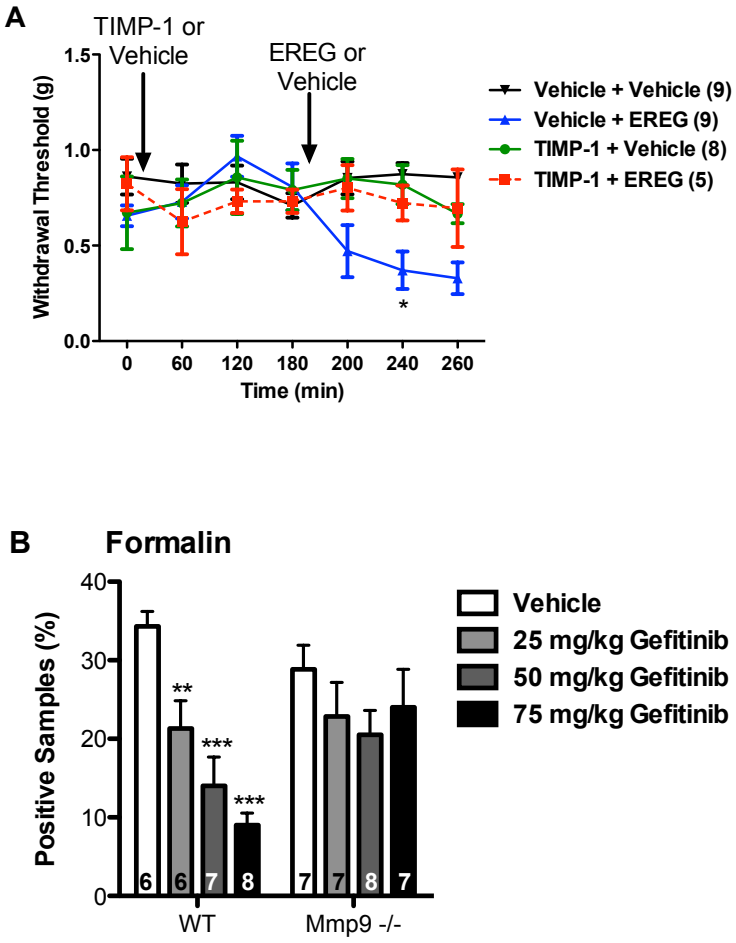
Supplemental Figure 3. **A**) QQ plot of TMD cases vs. supercontrols in OPPERA Caucasians. The SNPs from *EGFR* and *EREG* are labeled in red and green, respectively. **B)** Association of *EGFR* haplotypes with TMD. Forest plots depicting odds ratios (OR; with 95% confidence intervals) in three human chronic pain cohorts for individual *EGFR* 5' endohaplotypes (left) and 3' endohaplotypes (right) versus all others. The 5' haplotypes consist of SNPs rs759171 and rs4947963; the 3' haplotypes consist of SNPs rs845552, rs2740762, rs1140475. Complete information on haplotype association results is presented in **Supplementary Table 5**; haplotypes with the strongest contribution are presented here.



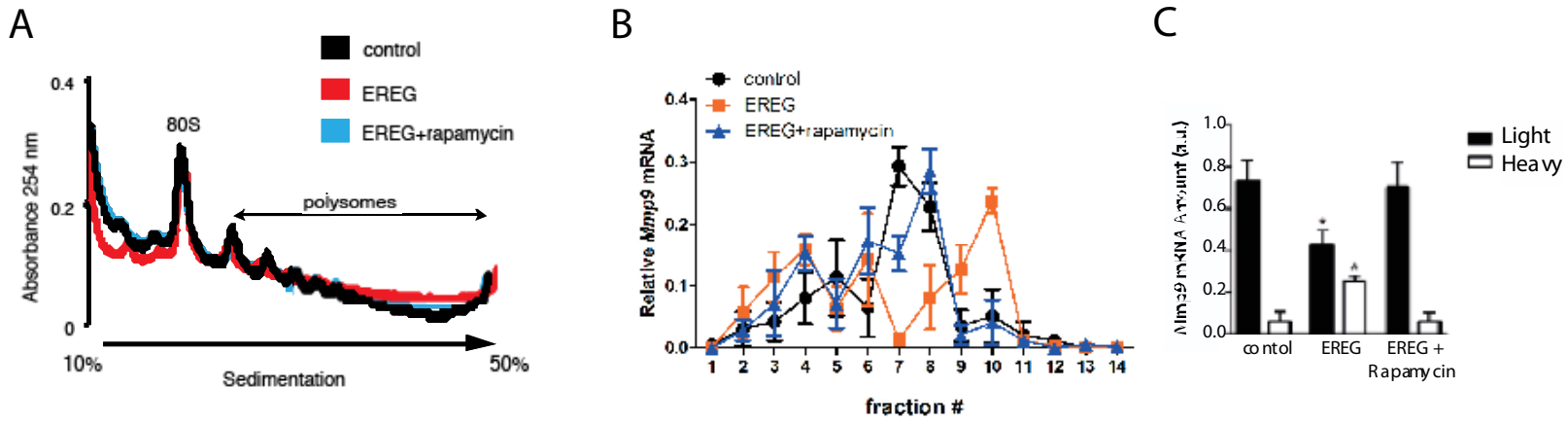
Supplementary Figure 4. (A and D) In the superficial dorsal horn of the spinal cord, EGFR-IR (green) was weakly expressed and observed as small dots. The staining for neurons labeled with NeuN (red) is shown in **B** and **E**. EGFR did not colocalize with neurons labeled with NeuN (**C and F**) suggesting that the source of EGFR in the spinal cord is non-neuronal.



Supplemental Figure 5. Inhibition of the ERK pathway produces analgesia, but does not block EREG hypersensitivity. (**A**) PD98059 (1 μg, i.t.), a MEK1/2 inhibitor, produces analgesia but does not block EREG induced hypersensitivity on the late phase of the formalin test (main effect of EREG: $F_{1,22}$=31.6, p<0.001; main effect of PD 98056: $F_{1,22}$=11.6, p=0.002). Bars represent mean ± SEM percentage of samples featuring licking/biting behavior. (**B**) PD98059 does not block EREG induced hypersensitivity on the von Frey test, but is slightly analgesic ($F_{3,18}$=31.8, p<0.001). Bars represent mean ± SEM area under the curve over the 60-min testing period for von Frey mechanical testing (at 0, 15, 30 and 60 min post-injection). Sample sizes are provided on graphs. *p<0.05, **p<0.01 increase compared to vehicle group. ^{o}p<0.05, ^{ooo}p<0.001 decrease compared to vehicle group.



Supplementary Figure 6. MMP-9 inhibition blocks EREG hypersensitivity, and *Mmp9* null mutant mice are less sensitive to the analgesic properties of gefitinib on the formalin test. (**A**) Pretreatment with TIMP-1 (4 pmol, i.t.), an endogenous inhibitor of MMP-9 prevents EREG-induced mechanical allodynia on the von Frey test (TIMP-1 x EREG x repeated measures interaction: $F_{6,162}$=2.6, p=0.02) without affecting mechanical sensitivity *per se*. Symbols represent mean ± SEM withdrawal threshold (g). (**B**) EGFR antagonist gefitinib produces dose-dependent analgesia in wildtype (*Mmp9*$^{+/+}$) but not *Mmp9* null mutant (*Mmp9*$^{-/-}$) mice (genotype x dose: $F_{3,48}$=3.2, p=0.03). Bars represent mean ± SEM percentage of samples featuring licking/biting behavior. Sample sizes are presented on graphs. *p<0.05, **p<0.01, ***p<0.001 compared with vehicle group using posthoc test for repeated measures (**A**) or Dunnett's case-comparison posthoc test (**B**).



Supplementary Figure 7. EREG stimulates *MMP-9* mRNA translation in an mTOR-dependent manner. **(A)** Polysome profiling of DRG lysates treated with vehicle, EREG (10 ng, i.t.) or EREG+rapamycin. Rapamycin (10mg/kg) was injected 20 min before EREG, and the lumbar DRG and spinal cord tissue were harvested 40 min after EREG injection. (**B**) Distribution of *Mmp9* mRNAs across sucrose gradient fractions prepared from DRG lysates (n=3, technical replicates). Fractions 5-14 are polysome fractions. (**C**) The relative amount of *Mmp9* mRNA in the light (5-9) and heavy (10-14) polysome fractions is quantified (*p<0.05 compared to analogous control condition). *Mmp9* mRNA co-sediments with heavier polysome fractions in EREG-treated DRG lysates, indicating increased rates of translation, and this effect is blocked by rapamycin.

Supplemental Tables.

Supplemental Table 1. Half-maximal analgesic doses (AD_{50}s) and 95% confidence intervals (95% CI) for EGFR inhibitor reversal of pain behavior on the late-phase of the formalin test. Morphine is presented for comparison purposes.

Drug	**AD_{50} (mg/kg)**	**95% CI (mg/kg)**
AG 1478	5.1	2.3–12.1
Gefitinib	14.1	8.3–24.2
Lapatinib	61	29.6–125
Morphine	4.0	1.9–8.5

Supplemental Table 2. Half-maximal analgesic doses (AD_{50}s) and 95% confidence intervals (95% CI) for EGFR inhibitor reversal of mechanical hypersensitivity after CFA (day 3 post-injection) and SNI (day 7 post-surgery). Doses are reported in mg/kg.

	CFA		SNI	
Drug	**AD_{50}**	**95% CI**	**AD_{50}**	**95% CI**
AG 1478	24	14–43	77	47–129
Gefitinib	37	18–78	195	40–1000
Lapatinib	55	34–88	111	57–217

Supplemental Table 3. Demographic characteristics of four human pain cohorts.

	OP-All		OP-SC		TMD		pre-OP
	cases	controls	cases	S-controls	cases	controls	cohort
N	166	1442	129	231	200	198	186
Female	83.1%	56.0%	100%	100%	100%	100%	100%
White	78.3%	52.6%	100%	100%	100%	100%	100%
Black	12.7%	29.7%					
Other/Refused	9.0%	17.7%					
Age (Mean, SD)	29.0 (8.0)	27.0 (7.7)	28.5 (8.0)	25.6 (6.7)	36.8 (12.2)	29.9 (11.0)	22.8 (4.7)

Abbreviations: OP-All: OPPERA study, all subjects; OP-SC: OPPERA study, "super-controls"; TMD: TMD case-control cohort; pre-OP: pre-OPPERA cohort. See **Online Methods** section for details.

Supplementary Table 4. Top-ranking *p* –values of cellular pathways associated with TMD in discovery cohort OPPERA cases vs. "supercontrols".

Index	Pathway	p-value
182	EGFR -> AP-1/ATF2 signaling	0.0013
188	EGFR/ERBB2 -> TP53 signaling	0.0042
175	GFR -> AP-1/CREB/CREBBP/ELK-SRF/MYC signaling	0.0052
179	EGFR -> CTNND signaling	0.0074
187	EGFR -> ZNF259 signaling	0.0074
183	EGFR/ERBB2 -> CTNNB signaling	0.0094
18	Adherens Junction Regulat on	0.0100
216	TGFBR -> AP-1 signaling	0.0103
82	ThrombinR -> AP-1/CREB/ELK-SRF/SP1 signaling	0.0110
109	VasopressinR1 -> CREB/ELK-SRF/AP-1/EGR signaling	0.0110
17	Focal Adhesion Regulation	0.0129
103	AdenosineR -> AP-1 signaling	0.0136
145	FibronectinR -> AP-1/ELK-SRF/SREBF signaling	0.0142
95	DopamineR2 -> AP-1/CREB/ELK-SRF signaling	0.0173
116	NeurotensinR -> ELK-SRF/AP-1/EGR signaling	0.0190
180	EGFR -> SMAD1 signaling	0.0197
185	EGFR/ERBB2 -> HIF1A signaling	0.0226
136	VasopressinR2 -> CREB/ELK-SRF/AP-1/EGR signaling	0.0234
10	Gonadotrope Cell Activat on	0.0259
128	EndothelineRa -> AP-1/CREB signaling	0.0335
151	ICAM1 -> AP-1/CREB/ELK-SRF signaling	0.0335
218	TGFBR -> ATF/GADD/MAX/TP53 signaling	0.0378
220	TGFBR -> MEF/MYOD/MYOG signaling	0.0378
177	GFR -> FOXO3A signaling	0.0401
178	GFR -> NCOR2 signaling	0.0402
245	TLR -> AP-1 signaling	0.0430
225	NGFR -> AP-1/CEBPB/CREB/ELK-SRF/TP53 signaling	0.0431
210	T-cell receptor -> AP-1 signaling	0.0447
238	EctodysplasinR -> AP-1 signaling	0.0447
198	VEGFR -> ATF/CREB/ELK-SRF signaling	0.0453
86	CCR5 -> TP53 signaling	0.0489
156	Notch -> TCF3 signaling	0.0490
191	FGFR-> RUNX2 signaling	0.0492
205	IGF1R -> MEF/MYOD/MYOG signaling	0.0506
235	TNFRSF1A -> AP-1/ATF/TP53 signaling	0.0539
236	TNFR -> AP-1/ATF/TP53 signaling	0.0539
203	IGF1R -> CEBPA/FOXO1A signaling	0.0563

				Association Analysis						Logistic Regression						
SNPs	**NSNP**	**NHAP**	**HAPLOTYPE**	**HAP_FREQ**	**F_A**	**F_U**	**CHISQ**	**DF**	**P_CHISQ**	**OR**	**STAT**	**P_LOG**	**LOG_OR**	**SE_LOG_OR**	**LB_OR**	**UB_OR**
rs759171|rs4947963	2	3	OMNIBUS	NA	NA	NA	6.54	2	0.04	NA	6.92	0.03				
rs759171|rs4947963	2	3	CC	0.35	0.35	0.35	0.01	1	0.92	1.01	0.01	0.93	0.01	0.12	0.80	1.27
rs759171|rs4947963	2	3	AT	0.13	0.16	0.10	5.86	1	0.02	1.71	5.92	0.02	0.54	0.22	1.11	2.63
rs759171|rs4947963	2	3	CT	0.52	0.49	0.55	3.00	1	0.08	0.77	3.24	0.07	-0.27	0.15	0.57	1.02
rs1140475|rs2740762|rs845552	3	4	OMNIBUS	NA	NA	NA	2.47	3	0.48	NA	2.18	0.54				
rs1140475|rs2740762|rs845552	3	4	TAG	0.13	0.14	0.13	0.57	1	0.45	1.17	0.61	0.44	0.16	0.20	0.79	1.74
rs1140475|rs2740762|rs845552	3	4	CAG	0.03	0.04	0.03	0.43	1	0.51	1.34	0.50	0.48	0.29	0.41	0.59	3.02
rs1140475|rs2740762|rs845552	3	4	CCG	0.32	0.30	0.35	2.06	1	0.15	0.81	1.87	0.17	-0.21	0.15	0.60	1.10
rs1140475|rs2740762|rs845552	3	4	CCA	0.50	0.51	0.49	0.35	1	0.55	1.11	0.50	0.48	0.10	0.15	0.83	1.48
rs759171|rs4947963	**2**	**3**	**OMNIBUS**	**NA**	**NA**	**NA**	**6.45**	**2**	**0.04**	**NA**	**6.37**	**0.04**				
rs759171|rs4947963	**2**	**3**	**CC**	**0.33**	**0.35**	**0.32**	**2.08**	**1**	**0.15**	**1.11**	**1.88**	**0.17**	**0.10**	**0.08**	**0.96**	**1.29**
rs759171|rs4947963	**2**	**3**	**AT**	**0.14**	**0.14**	**0.12**	**2.57**	**1**	**0.11**	**1.18**	**2.37**	**0.12**	**0.17**	**0.11**	**0.96**	**1.46**
rs759171|rs4947963	**2**	**3**	**CT**	**0.53**	**0.51**	**0.55**	**6.06**	**1**	**0.01**	**0.84**	**5.96**	**0.01**	**-0.18**	**0.07**	**0.73**	**0.97**
rs1140475|rs2740762|rs845552	**3**	**5**	**OMNIBUS**	**NA**	**NA**	**NA**	**10.32**	**4**	**0.04**	**NA**	**11.40**	**0.02**				
rs1140475|rs2740762|rs845552	**3**	**5**	**TAG**	**0.11**	**0.11**	**0.10**	**2.12**	**1**	**0.15**	**1.20**	**2.44**	**0.12**	**0.18**	**0.12**	**0.95**	**1.51**
rs1140475|rs2740762|rs845552	**3**	**5**	**CAG**	**0.04**	**0.03**	**0.05**	**8.26**	**1**	**0.00**	**0.54**	**9.54**	**0.00**	**-0.62**	**0.20**	**0.37**	**0.80**
rs1140475|rs2740762|rs845552	**3**	**5**	**CCG**	**0.34**	**0.34**	**0.33**	**0.41**	**1**	**0.52**	**1.06**	**0.56**	**0.46**	**0.06**	**0.08**	**0.91**	**1.23**
rs1140475|rs2740762|rs845552	**3**	**5**	**CAA**	**0.02**	**0.01**	**0.02**	**0.20**	**1**	**0.65**	**0.81**	**0.38**	**0.54**	**-0.21**	**0.35**	**0.41**	**1.60**
rs1140475|rs2740762|rs845552	**3**	**5**	**CCA**	**0.49**	**0.50**	**0.50**	**0.07**	**1**	**0.79**	**0.98**	**0.10**	**0.76**	**-0.02**	**0.07**	**0.85**	**1.13**
rs759171|rs4947963	2	3	OMNIBUS	NA	NA	NA	1.05	2	0.59	NA	1.60	0.45				
rs759171|rs4947963	2	3	CC	0.34	0.40	0.36	0.92	1	0.34	1.22	1.48	0.22	0.20	0.16	0.89	1.68
rs759171|rs4947963	2	3	AT	0.14	0.12	0.14	0.40	1	0.53	0.83	0.55	0.46	-0.19	0.25	0.51	1.36
rs759171|rs4947963	2	3	CT	0.51	0.48	0.50	0.25	1	0.62	0.89	0.52	0.47	-0.12	0.16	0.65	1.22
rs1140475|rs2740762|rs845552	3	5	OMNIBUS	NA	NA	NA	6.86	4	0.14	NA	13.00	0.01				
rs1140475|rs2740762|rs845552	3	5	TAG	0.11	0.15	0.09	5.80	1	0.02	2.22	9.00	0.00	0.80	0.27	1.32	3.74
rs1140475|rs2740762|rs845552	3	5	CAG	0.03	0.03	0.02	0.22	1	0.64	1.82	1.09	0.30	0.60	0.57	0.59	5.60
rs1140475|rs2740762|rs845552	3	5	CCG	0.34	0.36	0.37	0.13	1	0.72	0.95	0.08	0.78	-0.05	0.18	0.67	1.34
rs1140475|rs2740762|rs845552	3	5	CAA	0.02	0.02	0.01	0.31	1	0.58	1.86	0.62	0.43	0.62	0.79	0.40	8.70
rs1140475|rs2740762|rs845552	3	5	CCA	0.49	0.44	0.50	2.15	1	0.14	0.66	5.70	0.02	-0.42	0.17	0.47	0.93

Supplementary Table 5. Green cases = **TMD Case-Control Cohort (***200 cases, 198 controls), black cases* = **OPPERA Caucasians, Cases vs. Controls (***127 cases, 731 controls), red cases* = **OPPERA Caucasians, Cases vs. Supercontrols (***127 cases, 231 supercontrols.* Association analysis for black and red cases did not control for other covariates and logistic regression controlled for sex and site. **Abbreviations:** HAP_FREQ=overall frequency of haplotype (F from logistic regression output), F_A=frequency in affected (TMD cases), F_U=frequency in unaffected (TMD controls/supercontrols).