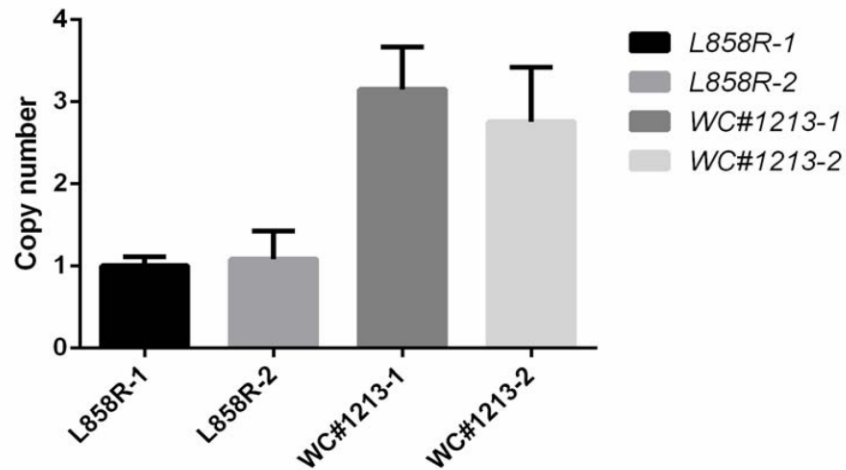


Overexpression of wildtype EGFR is tumorigenic and denotes a therapeutic target in non-small cell lung cancer

Supplementary Material



Supplementary figure 1

Founder line WC1213 harbors 3 copies of TetOnwtEGFR transgene

Tail DNA was isolated from our earlier established transgenic EGFR L858R mice (singlecopy) and WC1213 transgenic mice. Realtime PCR was carried out with primers indicated below. 2 mice each was used. Result indicated that WC1213 founder line harbored 3 copies of transgene.

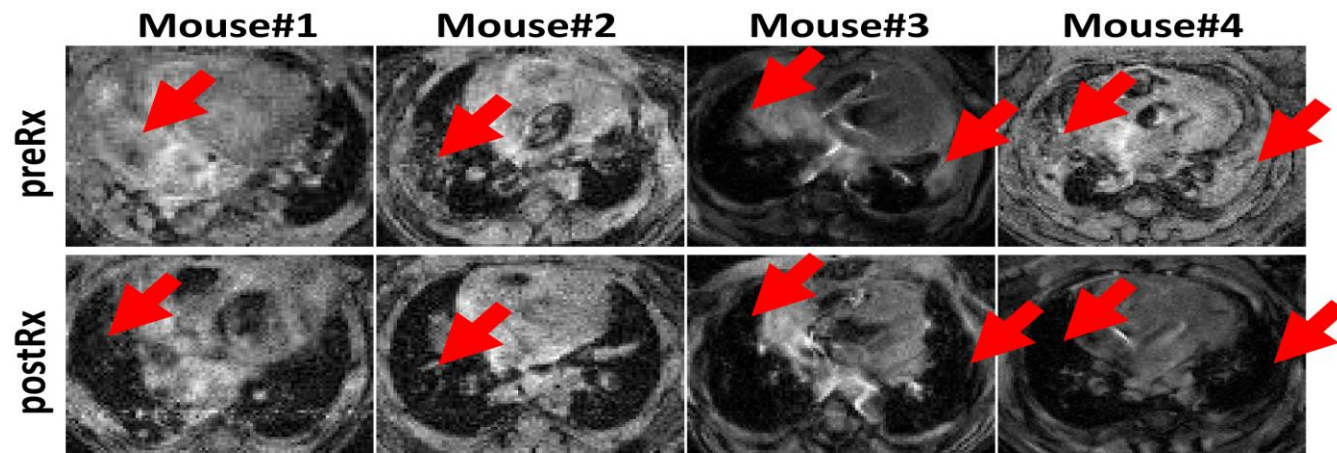
ATCCAGTGTGCCACTACATTG EGFRF TACTTCCAGACCAGGGTGTGTGTTGT ActinF acagcttctctttgatgtcagc ActinR
tgtgatggactccggagacgg



Supplementary figure 2

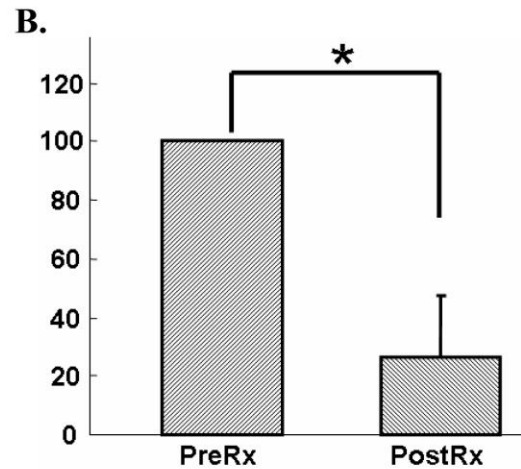
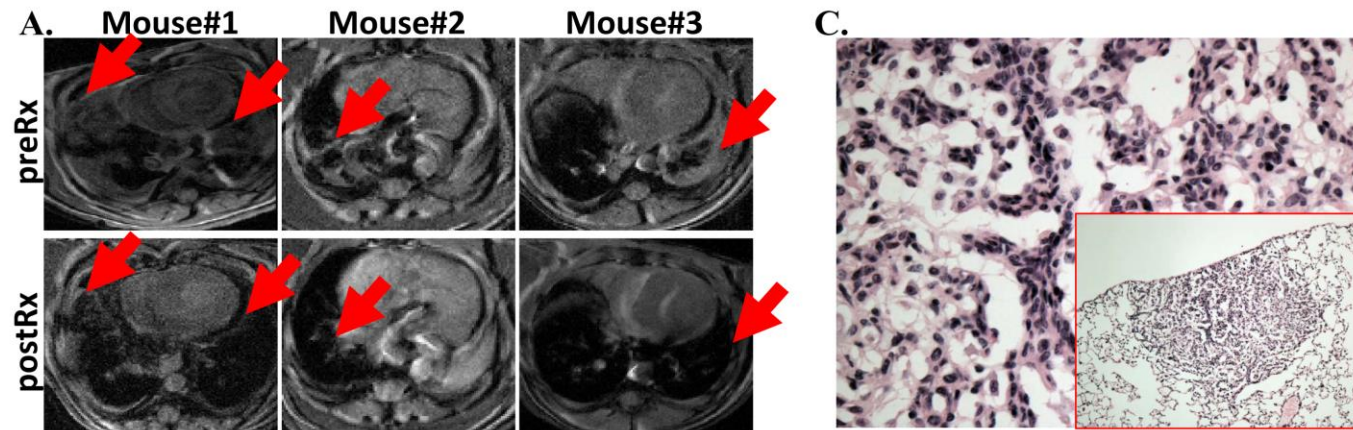
wtEGFR driven lung cancers depend on continuous expression of EGFR transgene for maintenance

CC10rtTA/Tet-OnEGFR bitransgenic mice were fed with doxycycline diet for around 8 months and then subjected to doxycycline withdrawal for 2 weeks. MRI images recorded before (upper panel) and after (lower panel) doxycycline withdrawal showed complete tumor regression. Tumor region highlighted with red arrow head.



Supplementary figure 3

Wt EGFR driven lung cancers are sensitive to erlotinib treatment CC10rtTA/TetO wt EGFR bitransgenic mice were fed with doxycycline diet for around 8 months and then treated with erlotinib for 2 weeks. MRI images recorded before (upper panel) and after (lower panel) erlotinib treatment showed dramatic tumor regression. Tumor region highlighted with red arrow head. Result revealed tumor regression in all 4 mice



Supplementary figure 4

Wt EGFR driven lung cancers are sensitive to cetuximab treatment

Three CC10rtTA/TetO wt EGFR bitransgenic mice were fed with doxycycline diet for around 8 months and then treated with cetuximab for 2 weeks. **A.** MRI images recorded before (upper panel) and after (lower panel) cetuximab treatment showed dramatic tumor regression. Tumor region highlighted with red arrow head. **B.** Quantification of tumor regression.

C. H&E analysis of the lung pathology revealed almost more histology with occasional areas of fibrosis indicating remodeling of original tumor area.

Supplementary table 1: OncoCarta™ Panel v1.0—mutations list**(total 19 oncogenes and 238 sites)**

oncogene (mutational sites)	Chromosomal locus	Mutational detail	
ABL1(14)	9q34.1	G250E, Q252H, Y253H, Y253F, E255K, E255V, D276G, F311L, T315I, F317L, M351T, E355G, F359V, H396R	
AKT1(7)	14q32.32	V461L, P388T, L357P, E319G, V167A, Q43X, E17K	
AKT2(2)	9q13.1-q13.2	S302G, R371H	
BRAF(24)	7q34	G464R, G464V/E, G466R, F468C, G469S, G469E, G469A, G469V, G469R, G469R, D594V/G, F595L, G596R, L597S, L597R, L597Q, L597V, T599I, V600E, V600K, V600R, V600L, K601N, K601E	
CDK(2)	12q14	R24C, R24H	

EGFR(43)	7p21	R108K, T263P, A289V, G598V, E709K/H, E709A/G/V, G719S/C, G719A, M766_A767insAI, S768I, V769_D770insASV, V769_D770insCV, D770_N771>AGG/N771>GF/N771T, D770_N771insG, V769_D770insASV, D770_N771insGF/D770_N771insGL, D770fs*61/D770_N771insAPW, P772_H773insV, H773N, H773_V774insNPH/PH/H, V774_C775insHV, T790M, L858R, L861Q, E746_A750del, E746_T751del, E746_T751>A/V/S, E746_S752>V/A/D/I, L747_E749del, L747S, L747_A750del, L747_A750>P, L747_T751del, L747_T751>P, L747_S752del, L747_P753>S/Q, A750P, T751A, T751P, T751I, S752F, S752_I759del, P753Q,
ERBB2(7)	17q21.1	L755P, G776S/LC, G776VC/VC,A775_G776insYVMA, P780_Y781insGSP,P780_Y781insGSP, S779_P780insVGS
FGFR1(2)	8p11.2-p11.1	S125L, P252T
FGFR3(5)	4p16.3	G370C, Y373C, A391E, K650Q/E, K650T/M
FLT3(3)	13q12	I836del, D835H/Y
HRAS(6)	11p15.5	G12V/D, G13C/R/S, Q61H/H, Q61L/R/P, Q61K
JAK(1)	9p24	V617F
KIT(27)	4q11-q12	D52N, Y503_F504insAY, W557R/R/G, V559D/A/G,V559I, V560D/G, K550_K558del, K558_V560del,K558_E562del, V559del, V559_V560del, V560del,Y570_L576del, E561K, L576P, P585P, D579del,K642E, D816V, D816H/Y, V825A, E839K, M552L,Y568D, F584S, P551_V555del, Y553_Q556del

KRAS(12)	12p12.1	G12C, G12R, G12S, G12V, G12D, G12A, G12F,G13V/D, A59T, Q61E/K, Q61L/R/P, Q61H/H	
MET(5)	7q31	R970C, T992I, Y1230C, Y1235D, M1250T	
NRAS(8)	1p13.2	G12V/A/D, G12C/R/S, G13V/A/D, G13C/R/S, A18T,Q61L/R/P, Q61H, Q61E/K	
PDGFRA(11)	7p22	V561D, T674I, F808L, D846Y, N870S, D1071N,D842_H845del, I843_D846del, S566_E571>K,I843_S847>T, D842V	
PIK3CA(13)	3q26.3	R88Q, N345K, C420R, P539R, E542K, E545K,Q546K, H701P, H1047R/L, H1047Y, R38H, C901F,M1043I	
RET(6)	10q11.2	C634R, C634W, C634Y, E632_L633del, M918T,A664D	

Supplementary Table 4 Clinicopathological characteristics of 61 NSCLC patients with wild-type EGFR

Age at diagnosis, y

mean (SD)	55.8±9.5
median	55.0
range	36.0-77.0

Sex, No. (%)

women	18 (29.5)
men	43 (70.5)

Pathology, No. (%)

ADC	48 (78.7)
non-ADC	13 (21.3)

Stage, No. (%)

IIIB	1 (1.6)
IV	60 (98.4)

Gefitinib, No. (%)

1st line	19 (31.1)
≥2nd line	42 (68.9)

EGFR h-score, No. (%)

EGFR positive	31 (50.8)
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EGFR negative	30 (49.2)
Gefitinib's best response, No. (%)	
PR	6(9.8%)
SD	32(52.5%)
PD	23(37.7%)

Abbreviations: PD-L1, programmed cell death-ligand 1; ADC, adenocarcinoma; non-ADC, non-adenocarcinoma; EGFR, epidermal growth factor receptor; EGFR-TKI, EGFR-tyrosine kinase inhibitor.

Supplementary Table 5 Multivariate analyses of PFS and OS

Parameter	PFS				OS			
	Multivariate analysis				Multivariate analysis			
	N	HR	95%CI	P-value	N	HR	95%CI	P-value
Age								
≤50y	1 17				1 17			
>50y	44	1.00 0	0.439-2.280	1.000	44	2.25 0	0.553-9.153	0.257
Sex								
female	1 18				1 18			
male	43	1.00 0	0.489-2.045	1.000	43	1.18 5	0.395-3.555	0.762
Pathology								
ADC	1 48				1 48			
non-ADC	13	1.00 0	0.366-2.734	1.000	13	1.31 6	0.306-5.567	0.712
Stage								
IIIB	1 1				1 1			
IV	60	1.00 0	0.000- 5118736	0.750	60	0.15 9	0.016-1.563	0.115

Gefitinib								
1st line	1	19			1	19		
≥2nd line	42	1.00 0	0.509-1.963	1.000	42	0.96 3	0.358-2.589	0.941
EGFR h-score								
negative	1	30			1	30		
positive	31	1.00 0	0.513-1.950	1.000	31	0.60 6	0.220-1.669	0.332

Abbreviations: OS, overall survival; PFS, progression-free survival; HR, hazard ratio; 95%CI, 95% confidence intervals;

ADC, adenocarcinoma; non-ADC, non-adenocarcinoma; EGFR, epidermal growth factor receptor; EGFR-TKI, EGFR-

tyrosine kinase inhibitor.

Supplementary Table 6 duration of response types among patients

Response type	N	PFS (months, mean \pm SD)
PR	6	12.77 \pm 10.72
SD	32	7.43 \pm 8.80
PD	23	1.32 \pm 1.23