Figure S1 Linear principal component analysis of plasma-derived exosomal RNA across 40 samples from NSCLC EGFR T790M patients receiving treatment with osimertinib.
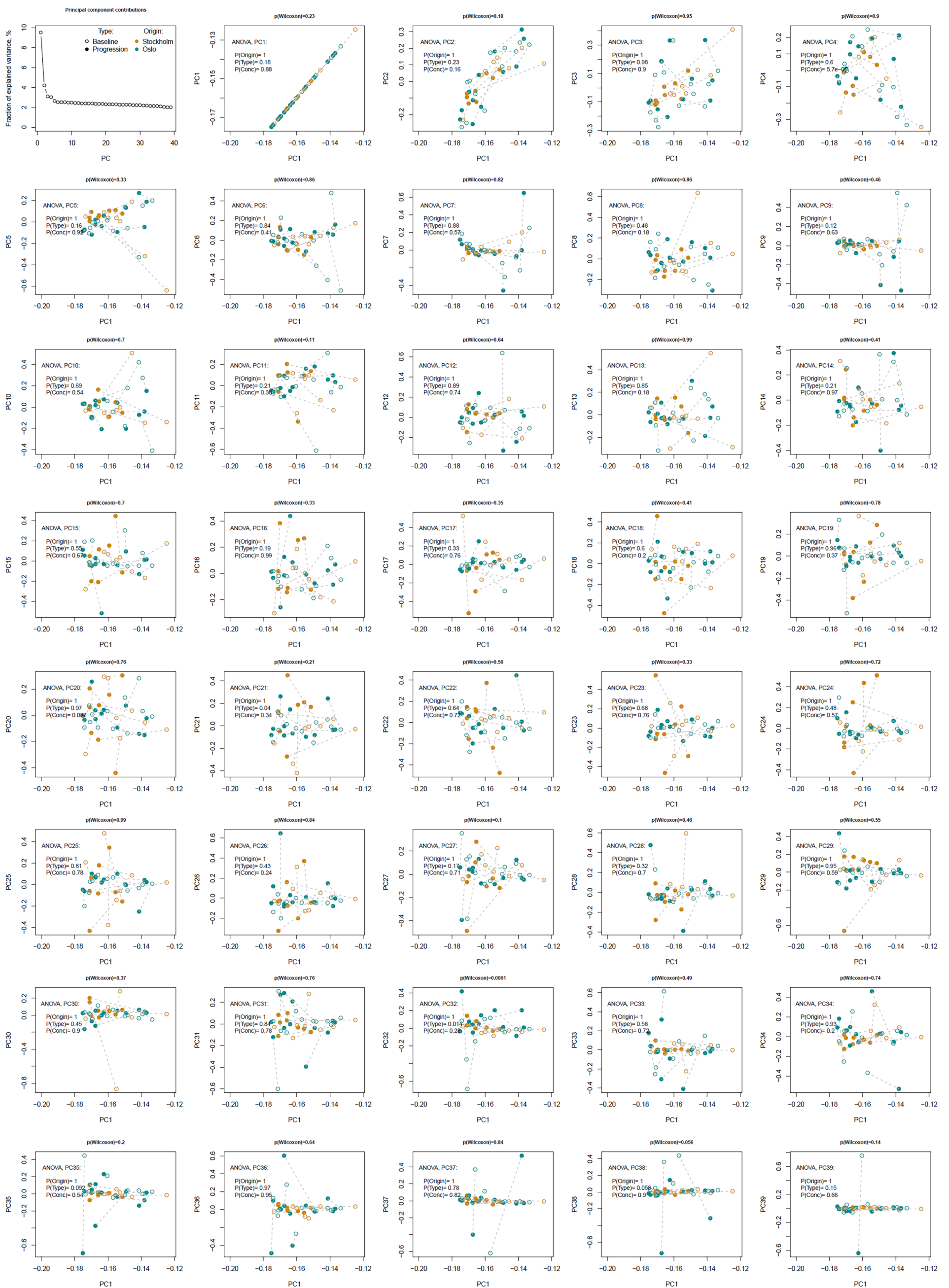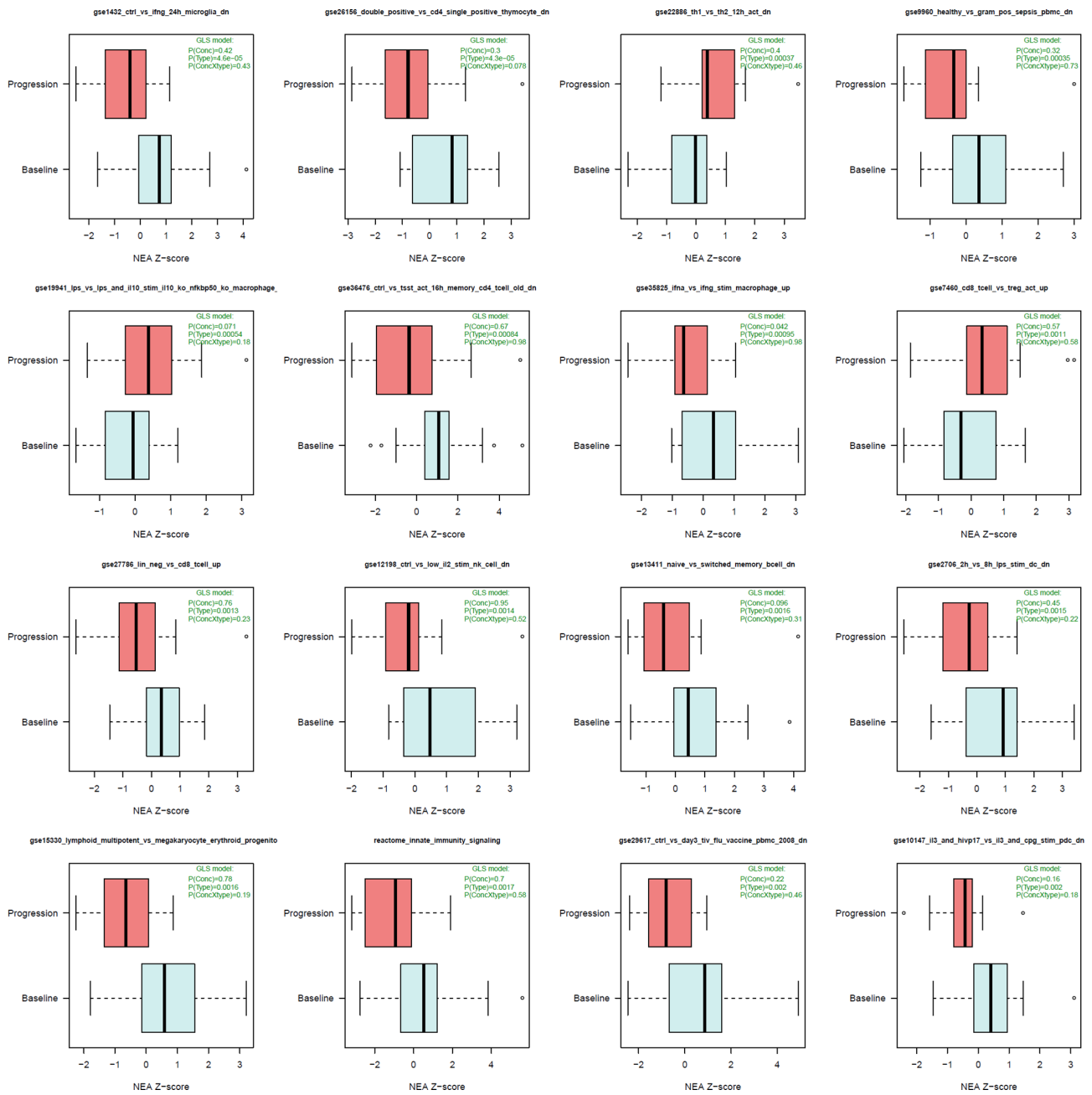
Figure S2 Pair-wise comparison of the 39 principal components.

Figure S3 Individual network enrichment analysis (NEA) displaying differentially activated pathways, in addition to GSE35825, with respect to 20 baseline AGS compared to progression samples from the same patient.

Table S1 Extended clinical parameters of each individual patient

Site	Progression	T790M-status	Sex	Age	Smoking status	PS	Histol	EGFR mut	PFS months
Helsinki	w16	mut	M	38	Never	1	AC	ex19	3.81
Helsinki	w24	mut	F	62	Never	1	AC	ex19	5.42
Helsinki	w84	mut	M	68	Former	1	AC	ex19	18.73
Linkøping	w32	mut	F	69	Former	2	AC	ex19	7.03
Oslo	w48	mut	F	53	Never	0	AC	ex19	11.3
Oslo	w32	mut	F	73	Current	1	AC	ex18	7.26
Helsinki	w32	mut	F	56	Former	1	AC	ex19	7.2
Linkøping	w24	mut	F	60	Former	1	AC	ex21	5.22
Linkøping	w48	mut	F	75	Former	1	AC	ex21	10.71
Oslo	w32	mut	M	43	Never	1	AC	ex19	7
Oslo	w48	mut	F	79	Former	1	AC	ex21	10.61
Oslo	w60	mut	F	59	Current	1	AC	ex21	13.14
Karolinska	w60	mut	F	75	former	0	AC	ex19	13.73
Karolinska	w32	mut	M	65	never	1	AC	ex19	7.36
Karolinska	w48	mut	F	76	former	0	AC	ex19	10.64
Karolinska	w60	mut	F	66	never	0	AC	ex19	12.88
Karolinska	w32	mut	M	78	never	0	AC	ex19	5.39
Karolinska	w60	mut	F	69	former	0	AC	ex19	14.23
Karolinska	w96	mut	F	61	never	0	AC	ex19	11.24
Karolinska	w16	mut	F	64	never	1	AC	ex19	3.68

EGFR, epidermal growth factor receptor; PFS, progression-free survival; AC, adenocarcinoma.

Table S2 Representation of cancer gene mRNA in transcriptomics datasets

Dataset	Source of material	Transcriptomics platform	Mean variability in numbers of genes with low or absent expression across chromosomes	Fraction of genes with low or absent expression across chromosomes, mean ± st.dev.
Clariom_D, patients	Blood plasma	Clariom_D	0.840	0.336±0.03
TCGA.LUAD (lung adenocarcinomas)	Primary tumors, fresh-frozen	RNA-seq	0.567	0.414±0.01
TCGA.LUSC (lung squamous cell sarcomas)	Primary tumors, fresh-frozen	RNA-seq	0.642	0.434±0.02
Clariom_D, cells	Cell culture	Clariom_D	0.521	0.188±0.06
CCLE, all cancer cell lines	Cell culture	RNA-seq	0.698	0.537±0.06
CCLE, cancer cell lines of lung origin	Cell culture	RNA-seq	0.645	0.427±0.05

For each of the human chromosomes, presence of cancer gene transcripts (386 genes found in the KEGG cancer-related pathways; codes KEGG#052*) was evaluated in each sample. Variability was expressed as variance of logit values $\log = \left(\dfrac{p}{1-p} \right)$, where p was the fraction of cancer genes with low or absent expression. The latter was defined as case of expression in a given sample significantly lower than the cohort mean (at Bonferroni-adjusted P value from t-test <0.01).

Table S3 Principal component analysis

PC	Eigenvalue's square root	Fraction of variance (%)	P.Type	P.Conc	P.Wilcoxon
1	2.416	9.50	0.181	0.8804	0.23
2	0.467	4.18	0.2269	0.161	0.18
3	0.26	3.12	0.9784	0.9033	0.95
4	0.243	3.02	0.5974	5.74E–06	0.9
5	0.185	2.63	0.1558	0.9167	0.33
6	0.169	2.52	0.8414	0.4125	0.86
7	0.167	2.50	0.8829	0.5654	0.82
8	0.166	2.49	0.4804	0.1768	0.86
9	0.164	2.48	0.1243	0.6255	0.46
10	0.161	2.45	0.6875	0.5357	0.7
11	0.158	2.43	0.2097	0.3815	0.11
12	0.155	2.41	0.8922	0.7445	0.64
13	0.154	2.40	0.8471	0.179	0.99
14	0.152	2.39	0.2138	0.9681	0.41
15	0.152	2.38	0.5484	0.6699	0.7
16	0.15	2.37	0.1854	0.9853	0.33
17	0.148	2.35	0.3339	0.7635	0.35
18	0.148	2.35	0.5988	0.2041	0.41
19	0.144	2.32	0.9628	0.3666	0.78
20	0.143	2.31	0.9695	0.08717	0.76
21	0.142	2.31	0.03978	0.3434	0.21
22	0.14	2.29	0.6438	0.7159	0.56
23	0.139	2.28	0.6827	0.7577	0.33
24	0.138	2.27	0.4772	0.5665	0.72
25	0.137	2.27	0.807	0.7787	0.99
26	0.136	2.26	0.4288	0.2359	0.84
27	0.135	2.25	0.1743	0.7122	0.1
28	0.132	2.22	0.3169	0.7037	0.46
29	0.131	2.21	0.9485	0.5893	0.55
30	0.129	2.19	0.4549	0.9016	0.37
31	0.126	2.17	0.8423	0.7758	0.76
32	0.124	2.16	0.01401	0.2774	0.0061
33	0.123	2.14	0.5784	0.7168	0.49
34	0.122	2.14	0.9263	0.2018	0.74
35	0.121	2.12	0.09156	0.5421	0.2
36	0.118	2.10	0.9653	0.9528	0.64
37	0.111	2.04	0.7752	0.8159	0.84
38	0.109	2.02	0.0585	0.9001	0.056
39	0.105	1.98	0.1471	0.6604	0.14

Table S4 Differentially expressed genes in Principal Component 32

Gene	Coefficient in PC32	P.Type	FDR.Type
PYY3	−0.166692315	2.20E–07	9.26E–06
ABCA2	−0.16120866	1.09E–06	1.53E–05
MT1L	0.285908111	8.00E–07	1.53E–05
PRODH2	−0.190786149	1.57E–06	1.65E–05
HMGB1P19	−0.175180873	5.95E–06	4.32E–05
MIR892B	0.1765141	6.17E–06	4.32E–05
OR56B2P	−0.36834929	1.55E–05	9.32E–05
GSG2	−0.322834092	3.73E–05	0.000195949
CHRNB2	−0.16535396	0.000120857	0.000391535
LINC00210	−0.216639704	0.000150933	0.000391535
MAP3K7CL	0.231942374	0.000158478	0.000391535
PGAM1P13	−0.175418078	0.000142233	0.000391535
PIGFP3	0.161248332	0.000119775	0.000391535
PREPL	0.444446945	0.000134088	0.000391535
SLC35A2	0.223094668	0.000128467	0.000391535
STT3B	0.472279445	9.62E–05	0.000391535
ZNF507	0.285862623	0.000131286	0.000391535
SCYL2	0.291051161	0.000174486	0.000407134
CHRNA3	0.167199573	0.000201859	0.000439237
DEFA3	−0.179454824	0.000228262	0.000439237
OR51Q1	−0.280672257	0.000230077	0.000439237
RPL35AP26	0.182099036	0.000218151	0.000439237
PBK	−0.4036554	0.000260394	0.000475502
ATP6V1B2	0.255478305	0.000289924	0.000507367
ATP1B1	0.384966212	0.000335871	0.000542561
SEC22A	0.221573944	0.000324379	0.000542561
ERCC4	0.171045665	0.000417382	0.000649261
FAM228B	0.215143778	0.000444358	0.000666537
GOLGA8B	−0.17285244	0.000471919	0.00068347
CSTF3	0.23327718	0.000578246	0.000792976
GABRG3	0.184682147	0.000585292	0.000792976
MOCS1	−0.177532192	0.000639775	0.000839704
PART1	0.193956082	0.000706772	0.000873072
PCED1A	0.262638443	0.000687793	0.000873072
ARHGAP42	0.265394995	0.000748461	0.000898154
MIR507	−0.233989024	0.000788235	0.000905413
RASSF5	0.185141951	0.000819183	0.000905413
ZNF786	0.228326367	0.000800372	0.000905413
LINC00314	0.289167794	0.000863075	0.000929465
CEP170P1	0.176312692	0.000905245	0.000931036
RNU6-271P	0.196029784	0.000908869	0.000931036
ERGIC2	0.395401712	0.000997413	0.000997413

Table S5 Differential gene expression in patients progressing on osimertinib and with an FDR <0.15

Gene_symbol	log2(fold_change).GE_patients	P(ConcXtype).GE_patients	FDR(Type).GE_patients
sworpaw	0.45	0.41	0.00026
luber	−0.36	0.1	0.00048
lawdarbo	0.65	0.15	0.00048
zochorbu	0.71	0.28	0.00048
PYY3	0.29	0.049	0.0023
wawleybo	0.98	0.23	0.0023
siyamu	0.35	0.059	0.0031
MT1L	−0.6	0.22	0.0064
ABCA2	0.25	0.04	0.0069
MKNK1	−0.88	0.58	0.0069
PRODH2	0.64	0.083	0.0083
lokar	0.5	0.66	0.0083
gortor	0.5	0.98	0.011
gertaw	0.58	1	0.011
UPP2-IT1	0.58	0.0015	0.016
sterbybo	0.67	0.23	0.016
terbeyby	0.55	0.23	0.016
IL17RA	−0.66	0.29	0.016
RASA1	−1.72	0.79	0.016
smyku	0.4	0.83	0.016
MIR892B	−0.5	0.37	0.017
choplorby	0.79	0.86	0.017
HMGB1P19	0.44	0.87	0.017
tenura	0.58	0.0067	0.018
CALM2P3	−0.73	0.7	0.022
ZNF17	0.83	0.74	0.022
plawwo	0.41	0.86	0.022
LIN9	−1.29	0.94	0.023
mortyby	0.47	0.15	0.024
tosoru	−0.24	0.043	0.026
gyveebo	0.56	0.84	0.026
OR56B2P	0.39	0.0083	0.029
MIR6818	0.46	0.32	0.029
tinima	0.41	0.75	0.029
slanabo	0.64	0.92	0.029
RGS18	−1.52	0.016	0.034
vasheybu	0.47	0.046	0.034
steywarby	0.48	0.73	0.034
APOBEC3D	−0.94	0.83	0.034
GTPBP2	−0.9	0.44	0.035
TRMT112P2	−0.53	0.3	0.039
floysterby	0.32	0.017	0.04
C18orf65	0.33	0.038	0.04
murera	0.57	0.42	0.04
44451	−0.35	0.07	0.041
WDR89	−0.66	0.2	0.043
spawglu	0.4	0.57	0.044
ODC1	−2.24	0.25	0.046
fustyby	0.54	0.66	0.046
ERICH6	−0.58	0.71	0.046
GSG2	0.46	0.25	0.047
LOC646903	0.32	0.13	0.052
HMGN2P31	−0.45	0.22	0.052
LINC00309	0.37	0.081	0.053
runiyo	0.52	0.067	0.057
geydoy	0.57	0.3	0.06
LINC01101	0.32	0.3	0.06
LINC00358	0.48	0.82	0.06
MICU2	−1.29	0.85	0.06
malor	0.39	0.9	0.06
LOC100131285	0.7	0.32	0.061
gluglybu	0.51	0.31	0.069
H2BFS	−1.11	0.00012	0.074
DDX39BP1	0.56	0.12	0.074
OR8B1P	0.67	0.5	0.074
BLOC1S2	−0.93	0.0088	0.076
EIF4BP1	−0.42	0.34	0.076
nanome	−0.6	0.022	0.078
bluspeeby	0.8	0.43	0.078
LOC606724	−0.41	0.71	0.078
sherbloyby	0.94	0.93	0.078
STT3B	−1.48	0.17	0.082
swawserbu	0.38	0.36	0.082
shermubu	0.51	0.95	0.082
homeobox.49	0.61	1	0.082
showy	0.34	0.23	0.084
RN7SKP294	0.32	0.44	0.084
nergorby	0.57	0.7	0.084
LOC643623	0.31	0.015	0.085
OR51A6P	0.38	0.017	0.085
geegee	0.64	0.3	0.085
HMGN2P32	−0.54	0.37	0.085
RP11-407N8.5	0.38	0.071	0.086
pleyjor	0.31	0.2	0.086
PIGFP3	−0.39	0.099	0.089
CHRNB2	0.38	0.48	0.089
AKAP3	0.74	0.16	0.09
skawspybu	0.42	0.2	0.09
SLC35A2	−0.38	0.33	0.092
PREPL	−1.39	0.24	0.093
ZNF507	−0.78	0.64	0.093
LINC01000	0.5	0.74	0.093
PGAM1P13	0.33	0.77	0.097
MAP3K7CL	−2.03	0.038	0.1
LOC105373185	0.2	0.2	0.1
wawter	0.3	0.42	0.1
C7orf50	−0.31	0.46	0.1
LINC00210	0.39	0.64	0.1
LOC100507443	0.24	0.65	0.1
nawmobu	0.51	0.7	0.1
HMGN2	−0.65	0.0028	0.11
SCYL2	−1.49	0.022	0.11
glarbleeby	0.47	0.081	0.11
RN7SL327P	0.35	0.14	0.11
RN7SL489P	0.35	0.14	0.11
RN7SL853P	0.35	0.14	0.11
OTUD3	0.71	0.16	0.11
S100A5	0.27	0.28	0.11
METTL4	−0.76	0.37	0.11
F13B	−0.52	0.52	0.11
CHRNA3	−0.32	0.66	0.11
rorplarby	0.63	0.73	0.11
TRBV7-7	0.28	0.76	0.11
RPL17P49	−0.44	1	0.11
sarstubu	0.53	0.00042	0.12
OR51Q1	0.64	0.006	0.12
C2orf88	−0.81	0.012	0.12
RPL35AP26	−0.3	0.023	0.12
yosoyu	0.51	0.088	0.12
NPC1	−0.58	0.31	0.12
DEFA3	1.09	0.37	0.12
GPR50	0.41	0.41	0.12
MAGED2	−0.69	0.59	0.12
LOC257396	0.17	0.69	0.12
spodu	0.44	0.78	0.12
nawblabu	0.33	0.79	0.12
LOC730100	0.25	0.83	0.12
shersoby	0.43	0.96	0.12
ELOVL7	−1.79	0.00096	0.13
KIAA1383	0.46	0.019	0.13
wordee	0.51	0.11	0.13
kleylaw	0.61	0.16	0.13
MIR4524B	0.7	0.26	0.13
OR4C13	−0.28	0.31	0.13
fawwee	0.44	0.53	0.13
timemi	−0.51	0.57	0.13
varsperbu	0.48	0.61	0.13
PBK	0.46	0.62	0.13
yanaro	0.39	0.69	0.13
skoplorbu	0.5	0.72	0.13
ATP6V1B2	−1.52	0.91	0.13
dorkluby	0.48	0.017	0.14
DDX11L2	−1.39	0.041	0.14
DDX11L10	−1.49	0.06	0.14
toybo	0.63	0.11	0.14
skugybu	−0.23	0.12	0.14
tukly	−0.48	0.14	0.14
toykoy	0.37	0.14	0.14
ATP1B1	−1.01	0.16	0.14
SEC22A	−0.58	0.22	0.14
NUPR2	−0.26	0.3	0.14
fawchy	−0.27	0.41	0.14
korboybo	0.4	0.69	0.14
steymoybo	0.58	0.69	0.14
CCT8L1P	−0.21	0.79	0.14
slorfer	0.44	0.86	0.14
MLXIP	−0.36	0.86	0.14
kuchoyby	0.8	0.91	0.14
RN7SL70P	−0.34	0.98	0.14
spudu	0.35	0.98	0.14

Table S6 Significant pathways in the overall NEA approach

FGS (pathway)	No.of.links.AGS-FGS	NEA.z-score	NEA.P-value	NEA.FDR
glypican_pathway	322	7.681	1.60E-14	1.10E-12
glypican_1_network	307	8.858	8.20E-19	9.60E-17
erbb_receptor_signaling_network	300	8.166	3.20E-16	2.90E-14
internalization_of_erbb1	290	8.028	9.90E-16	8.40E-14
proteoglycan_syndecan-mediated_signaling_events	272	8.601	7.90E-18	8.40E-16
trail_signaling_pathway	258	8.229	1.90E-16	1.80E-14
all623_ding2008	256	12.578	2.80E-36	1.30E-33
ifn-gamma_pathway	252	8.269	1.30E-16	1.30E-14
syndecan-1-mediated_signaling_events	251	8.051	8.20E-16	7.10E-14
signaling_events_mediated_by_hepatocyte_growth_factor_receptor_ (c-met)	249	8.14	3.90E-16	3.50E-14
signaling_events_mediated_by_focal_adhesion_kinase	244	8.036	9.30E-16	8.00E-14
class_i_pi3k_signaling_events	237	8.138	4.00E-16	3.60E-14
class_i_pi3k_signaling_events_mediated_by_akt	205	7.496	6.60E-14	4.40E-12
endothelins	186	7.968	1.60E-15	1.30E-13
kegg_05200_pathways_in_cancer	148	6.982	2.90E-12	1.60E-10
kegg_04010_mapk_signaling_pathway	132	7.111	1.20E-12	6.80E-11
reactome_hemostasis	128	7.476	7.60E-14	5.10E-12
hs_egfr1_signaling_pathway_wp437_35716.txt	124	8.39	4.90E-17	4.80E-15
hs_mapk_signaling_pathway_wp382_38878.txt	108	7.82	5.30E-15	4.10E-13
hs_insulin_signaling_wp481_38887.txt	90	8.345	7.10E-17	6.90E-15
hs_il-6_signaling_pathway_wp364_35645.txt	86	7.023	2.20E-12	1.20E-10
kegg_04012_erbb_signaling_pathway	84	7.397	1.40E-13	9.10E-12
hs_calcium_regulation_in_the_cardiac_cell_wp536_38956.txt	79	6.9	5.20E-12	2.80E-10
kegg_04530_tight_junction	75	9.733	2.20E-22	3.60E-20
kegg_05214_glioma	70	8.006	1.20E-15	1.00E-13
hs_g_protein_signaling_pathways_wp35_35311.txt	68	7.934	2.10E-15	1.70E-13
biocarta_biopeptides_pathway	64	9.129	6.90E-20	9.00E-18
kegg_04540_gap_junction	57	7.227	5.00E-13	3.00E-11
kegg_05223_non-small_cell_lung_cancer	56	6.899	5.20E-12	2.80E-10
biocarta_pdgf_pathway	52	7.601	2.90E-14	2.10E-12
biocarta_egf_pathway	51	6.944	3.80E-12	2.10E-10
hs_signal_transduction_of_s1p_receptor_wp26_38752.txt	25	7.513	5.80E-14	3.90E-12
kegg_00512_o-glycan_biosynthesis	14	10.232	1.40E-24	2.80E-22

Table S7 Significant pathways in the individual NEA approach

Gene_set	log2(fold_change)	p(ConcXtype)	FDR(Type)
gse26156_double_positive_*vs.*_cd4_single_positive_thymocyte_dn	1.26	0.078	0.037
gse1432_ctrl_*vs.*_ifng_24h_microglia_dn	1.21	0.43	0.037
gse9960_healthy_*vs.*_gram_pos_sepsis_pbmc_dn	0.84	0.73	0.13
gse22886_th1_*vs.*_th2_12h_act_dn	-0.89	0.46	0.13
gse19941_lps_*vs.*_lps_and_il10_stim_il10_ko_nfkbp50_ko_macrophage_up	-0.61	0.18	0.17
gse36476_ctrl_*vs.*_tsst_act_16h_memory_cd4_tcell_old_dn	1.49	0.98	0.18
gse35825_ifna_*vs.*_ifng_stim_macrophage_up	0.94	0.98	0.18
gse7460_cd8_tcell_*vs.*_treg_act_up	-0.62	0.58	0.19
gse27786_lin_neg_*vs.*_cd8_tcell_up	0.82	0.23	0.21
gse12198_ctrl_*vs.*_low_il2_stim_nk_cell_dn	1.07	0.52	0.21
gse2706_2h_*vs.*_8h_lps_stim_dc_dn	1.04	0.22	0.21
gse13411_naive_*vs.*_switched_memory_bcell_dn	0.93	0.31	0.21
gse15330_lymphoid_multipotent_*vs.*_megakaryocyte_erythroid_progenitor_up	1.23	0.19	0.21
reactome_innate_immunity_signaling	1.56	0.58	0.22
gse10147_il3_and_hivp17_*vs.*_il3_and_cpg_stim_pdc_dn	0.91	0.18	0.23
gse29617_ctrl_*vs.*_day3_tiv_flu_vaccine_pbmc_2008_dn	1.3	0.46	0.23

Table S8 Cross-validation of significant findings

	Patient cohort	In-house cell panel	CCLE
Patient cohort	GE, LM PA-1: 128 DE RNAs NEA(individual), LM PA-1: 16 DA FGS NEA(overall): 33 enriched pathways	NEA(individual): P(H-B) = 0.06 NEA(overall): P(H-B) = 0.011	NEA(individual): P(H-B) = 0.022
In-house cell panel	GE: p(H-B) = 0.0012	GE, LM CL-1: 16 DE RNAs NEA(individual), LM CL-1: 0 DA FGSs NEA(overall): 64 enriched pathways	NEA(individual): P(H-B)=0.0023
CCLE	GE: P(H-B) = 0.0002	GE: P(H-B) = 1.4e–25	GE, LM CCLE-1: 125 DE genes NEA(individual), LM CCLE-1: 0 DA FGS

GE, gene/RNA level analysis; NEA, pathway level analysis; LM, linear model; DE, differentially expressed; DA, differentially activated; P(H-B), P value from Fisher's exact text tried at a series of P value cutoffs of increasing strength, adjusted for multiple testing by Holm-Bonferroni method.