# Supplementary Materials for

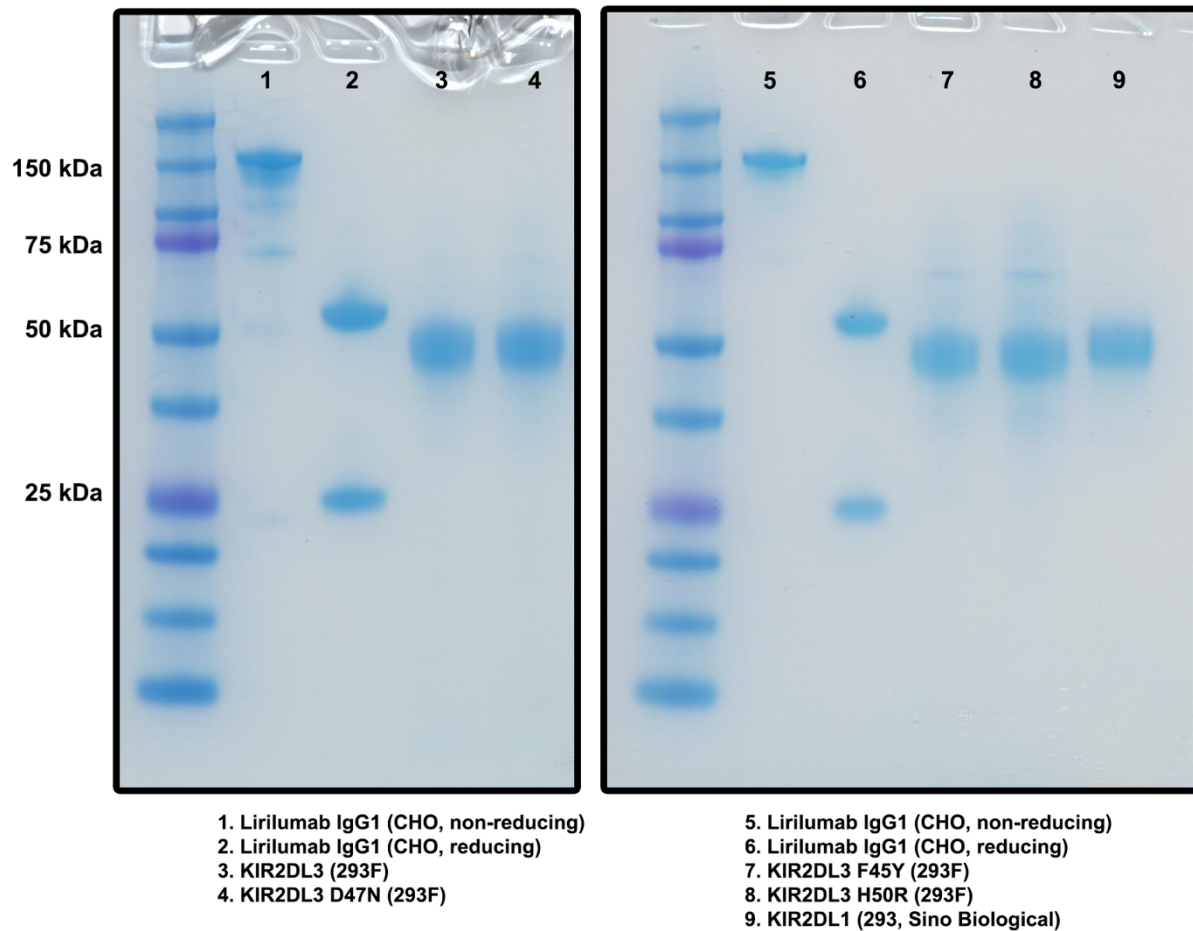## Structural basis for the activity and specificity of the immune checkpoint inhibitor lirilumab

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**Includes:**

Figs. S1
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**1. Lirilumab IgG1 (CHO, non-reducing)**
**2. Lirilumab IgG1 (CHO, reducing)**
**3. KIR2DL3 (293F)**
**4. KIR2DL3 D47N (293F)**

**5. Lirilumab IgG1 (CHO, non-reducing)**
**6. Lirilumab IgG1 (CHO, reducing)**
**7. KIR2DL3 F45Y (293F)**
**8. KIR2DL3 H50R (293F)**
**9. KIR2DL1 (293, Sino Biological)**

**Fig. S1: SDS-PAGE gels showing lirilumab IgG1, KIR receptors used for BLI assays.** Lirilumab IgG1 was purified from CHO-S cell supernatant using protein A resin. KIR receptors were purified from HEK293F supernatant using EDTA-resistant Ni-NTA followed by size exclusion chromatography.

**Table S1**. BLI kinetics model parameters for binding between lirilumab (IgG1) and KIR2DL1, KIR2DL3, and KIR2DL3 variants.

| | $K_D$ (nM)[a] | $R^2$ | $X^2$ | $k_a$ (1/Ms) | $k_d$ (1/s) |
|---|---|---|---|---|---|
| | | | Model Fit Parameters | | |
| KIR2DL3 | 1.64 | 0.9952 | 0.5141 | 2.240E05 | 3.663E-04 |
| | 2.83 | 0.9954 | 0.3523 | 2.197E05 | 6.219E-04 |
| | 3.78 | 0.9959 | 0.1989 | 2.363E05 | 8.920E-04 |
| KIR2DL1 | 11.5 | 0.9912 | 1.0485 | 8.319E04 | 9.537E-04 |
| | 9.6 | 0.9932 | 0.7627 | 1.038E05 | 9.946E-04 |
| | 8.8 | 0.9942 | 0.6410 | 1.074E05 | 9.475E-04 |
| KIR2DL3 F45Y | 10.25 | 0.9932 | 0.6602 | 1.218E05 | 1.249E-03 |
| | 10.8 | 0.9943 | 0.4065 | 1.437E05 | 1.554E-03 |
| | 8.5 | 0.9895 | 0.3643 | 1.705E05 | 1.450E-03 |
| KIR2DL3 D47N[b] | 1400 | 0.9991 | 0 | -- | -- |
| | 1900 | 0.9973 | 0.0001 | -- | -- |
| KIR2DL3 H50R | 7.98 | 0.9795 | 2.12 | 2.442E05 | 1.948E-03 |
| | 10.6 | 0.9818 | 1.311 | 2.308E05 | 2.437E-03 |
| | 15 | 0.9774 | 2.3512 | 2.116E05 | 3.175E-03 |

[a]$K_D$ determined using Octet Data Analysis HT software's 1:1 binding model with the 'global' fit setting and using at least 4 curves for each experiment
[b]$K_D$ determined using a steady state binding model due to poor fitting of the 1:1 model

**Table S2.** High frequency (>5%) inhibitory KIR2D receptor missense variants and other variants of interest

| Var#[a] | KIR | Variant accession number | Genome location (GRCh38.p14) | Mutation | Minor Allele Frequency, Global (1000genomes_30x) |
|---|---|---|---|---|---|
| | KIR2DL1 | rs666590 | 54775402 | A>G = H182R | 0.291 |
| | KIR2DL1 | rs147072532 | 54775344 | G>A = D163N | 0.277 |
| | KIR2DL1 | rs111799279 | 54775317 | C>A = P154T | 0.276 |
| | KIR2DL1 | rs11673144 | 54775198 | T>C = L114P | 0.266 |
| | KIR2DL1 | rs199644153 | 54775213 | T>A = L119N | 0.261 |
| | KIR2DL1 | rs597681 | 54778656 | A>G = K216E | 0.220 |
| | KIR2DL1 | rs687885 | 54773593 | G>T = V90L | 0.210 |
| | KIR2DL1 | rs574769826 | 54775225 | A>G = N123S | 0.199 |
| Var13 | KIR2DL1 | rs687485 | 54773534 | C>A = T70K | 0.194 |
| | KIR2DL1 | rs2916003 | 54775357 | G>A = G167D | 0.179 |
| | KIR2DL1 | rs200746024 | 54775315 | G>T = G153V | 0.158 |
| | KIR2DL1 | rs538559888 | 54775318 | C>G = P154R | 0.155 |
| | KIR2DL1 | rs34721508 | 54783002 | C>T = R245C | 0.155 |
| Var8 | KIR2DL1 | rs35509911 | 54773372 | G>C = R16P | 0.155 |
| | KIR2DL1 | rs62121640 | 54775329 | G>A = G158R | 0.151 |
| | KIR2DL1 | rs2756923 | 54783763 | A>G = T312A | 0.151 |
| | KIR2DL1 | rs2304224 | 54769863 | Signal sequence | 0.146 |
| | KIR2DL1 | rs202205602 | 54775305 | C>T = L150F | 0.065 |
| Var10 | KIR2DL1 | rs375476159 | 54773473 | C>T = R50C | No data |
| Var9 | KIR2DL1 | rs1481853508 | 54773452 | G>A = G43R; G>C = G43R | No data |
| Var11 | KIR2DL1 | rs760965171 | 54773494 | G>C = D57H | No data |
| Var12 | KIR2DL1 | rs765722009 | 54773519 | C>T = S65F | No data |
| | KIR2DL3 | rs3810343 | 54738571 | Signal sequence | 0.361 |
| Var2 | KIR2DL2/3 | rs35719984 | 54742075 | C>G = Q35E | 0.340 |
| | KIR2DL3 | rs1049267 | 54752446 | G>A = R297H; G>C = R297P | 0.236 (A) 0.160 (C) |
| | KIR2DL2/3 | rs200686594 | 54743929 | C>T = R148C | 0.194 |
| | KIR2DL2/3 | rs150145497 | 54744086 | C>T = T200I | 0.149 |
| Var4 | KIR2DL2/3 | rs78713511 | 54742106 | T>A = F45Y | 0.135 |
| | KIR2DL2/3 | rs1555897648 | 54742019 | C>G = P16R | 0.134 |
| | KIR2DL2/3 | rs201504521 | 54743909 | G>C = R141T | 0.120 |
| | KIR2DL3 | rs35861855 | 54747356 | C>T = P208L | 0.115 |
| | KIR2DL2/3 | rs200359536 | 54744000 | C>A = H171N | 0.096 |
| | KIR2DL2/3 | rs200426472 | 54743878 | C>T = R131W | 0.096 |
| | KIR2DL3 | rs2365227 | 54752222 | T>C = V255A | 0.085 |
| | KIR2DL3 | rs4020198 | 54752265 | G>C = R279S | 0.079 |
| Var7 | KIR2DL2/3 | rs138897134 | 54742121 | A>G = H50R | 0.057 |
| Var1 | KIR2DL2/3 | rs202032116 | 54742004 | T>G = L11R | 0.053 |
| Var3 | KIR2DL2/3 | rs76843526 | 54742094 | G>C = R41T | 0.006 |
| Var6 | KIR2DL2/3 | rs528413442 | 54742111 | G>A = D47N | 0.001 |
| Var5 | KIR2DL2/3 | rs145638569 | 54742110 | G>C = K46N | 0.0002 |

[a]Var#s correspond to those listed in Fig. 4.